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Effects of supplemental food on the behavior, aggression, and paternity status of male Indigo Buntings (*Passerina cyanea*)

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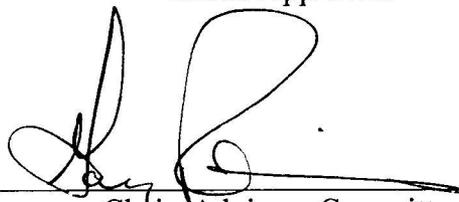
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EFFECTS OF SUPPLEMENTAL FOOD ON THE BEHAVIOR, AGGRESSION, AND
PATERNITY STATUS OF MALE INDIGO BUNTINGS (PASSERINA CYANEA)

By

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Thesis Approved:



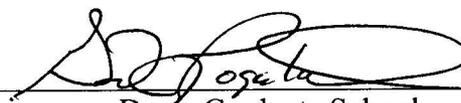
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PATERNITY STATUS OF MALE INDIGO BUNTINGS (PASSERINA CYANEA)

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ABSTRACT

Breeding is an energetically costly activity for birds. If energy-limited, birds may alter their time budgets, reducing time spent in some activities and spending more time in others. To date, no experimental study has investigated the possible effect of food availability on male mate guarding behavior. Additionally, previous results from food supplementation studies are mixed. My objectives were to determine how food supplementation might influence the breeding behavior of male Indigo Buntings (*Passerina cyanea*). I predicted that, compared to non-supplemented males, food-supplemented males would: 1) spend less time foraging, 2) spend more time singing, 3) spend more time mate guarding, 4) respond more aggressively to a simulated intruder (i.e. playback of conspecific songs in their territories), and 5) have fewer EPY in their nests. Behavioral observations were conducted at the Miller-Welch/Central Kentucky Wildlife Management Area from 8 May – 15 August 2009. Territories of male buntings (N = 30) in my study area were randomly selected for either food supplementation (n = 8) or controls (n = 22). During time budget observations (20 min/day/focal male), all behaviors were noted. To quantify male aggression, playback experiments were conducted on a subset of six food-supplemented males and six non-food-supplemented males, when numerous aggressive-like behaviors were noted. To determine paternity, blood samples were collected from adults and nestlings at 19 nests. DNA was extracted, amplified, and hand-scored on polyacrylamide gels. Males with feeders in their territories spent significantly less time foraging and significantly more time chipping than males without feeders in their territories. Time spent singing and mate guarding was not

statistically different between treatment and control males. Differences in aggressive behaviors and the proportion of EPY approached significance with those males with feeders in their territories exhibiting more aggressive-like behaviors and having more EPY in their nests. Of 16 broods, 12 (75%) had at least one EPY and, in those broods, 24 of 43 nestlings (56%) were EPY. My results suggest that when provided with supplemental food, males can spend less time foraging, and in turn, will have more time and energy to devote to other activities. The presence of supplemental food however, has appeared to increase the rates of intruding, neighboring males. When neighboring conspecifics are drawn to territories with supplemental food, more EPC opportunities exist for females on supplemented territories, therefore leading to a greater proportion of EPY for those females and suggesting that female buntings are not using food as an indicator of habitat or male quality. Mate guarding did not act to fully protect paternity and regardless of time spent mate guarding, females, whether mated to food-supplemented males or not, were not guarded for the majority of the time, thereby permitting ample opportunities to seek EPCs themselves. In addition, female buntings do not risk the loss of male provisioning assistance by seeking EPCs.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. METHODS	4
III. RESULTS	13
IV. DISCUSSION	19
LITERATURE CITED	28

LIST OF TABLES

TABLE	PAGE
1. Five microsatellite loci used in paternity analysis, sequences, number of alleles, observed heterozygosity, and the probability of excluding an unrelated offspring assuming the first parent's genotype is known, from Indigo Buntings at Miller-Welch/Central Kentucky Wildlife Management Area in Madison County, Kentucky	11
2. Status of all individuals that visited feeders	14
3. Comparison of mean responses of male Indigo Buntings with (n = 5) and without feeders (n = 7) in their territories to playback of conspecific song	17

LIST OF FIGURES

FIGURE	PAGE
1. Effect of supplemental food on the behaviors of male Indigo Buntings with (n = 8) and without feeders (n = 22) in their territories (mean time spent foraging, singing, mate guarding, chipping, fighting, and perching).....	15
2. Effect of supplemental food on the behaviors of male Indigo Buntings observed at (n = 15) and not observed at feeders (n = 15; mean time spent foraging, singing, mate guarding, chipping, fighting, and perching).....	16
3. Effect of supplemental food on male Indigo Bunting mean proportion of extra-pair young in nests on territories with (n = 8) and without feeders (n = 22)	18
4. Comparing male extra-pair paternity data for three different Indigo Bunting populations: Cass County, Michigan (Westneat 1987), Orange County, North Carolina (Westneat 1990), and Madison County, Kentucky (this study).....	25

CHAPTER I

INTRODUCTION

Breeding birds require food and energy for egg production, territory defense, nestling provisioning, and other associated activities (Lack 1947, Nur 1988, Visser and Lessells 2001, Williams 2005, Robb et al. 2008). When food is abundant, breeding birds may be able to spend less time foraging (Clifford and Anderson 2001, Fleischer et al. 2003) and more time engaged in other activities. Alternately, when food is limited, tradeoffs may be necessary. Birds may alter their time budgets, forgoing or reducing the time spent in some activities and spending more time foraging.

Several investigators have examined the possible effects of differences in food availability on avian breeding behavior by providing supplemental food. For example, Strain and Mumme (1988) found that male Carolina Wrens (*Thryothorus ludovicianus*) sang at higher rates when provided with supplemental food. Similar results have been reported for other species where males were provided with supplemental food, including Black Redstarts (*Phoenicurus ochruros*; Cucco and Malacarne 1997), Pied Flycatchers (*Ficedula hypoleuca*; Alatalo et al. 1990), and Savannah Sparrows (*Passerculus sandwichensis*; Reid 1987). Because singing plays a role in mate attraction and territory defense, the increased singing rates of food-supplemented males may help insure paternity if females perceive such males as being of higher quality and, as a result, are less likely to engage in extra-pair copulations (EPCs). In addition, increased singing rates may reduce the likelihood of territorial intrusions by conspecific males seeking EPCs.

Supplemental food may also influence the mating strategies of females in other ways. For example, O'Brien and Dawson (2011) found that multi-brooded female Mountain Bluebirds (*Sialia currocooides*) provided with supplemental food throughout their first breeding attempts were less likely to have extra-pair young (EPY) in their second broods than non-supplemented females. Similarly, female House Sparrows (*Passer domesticus*) provided with food prior to breeding had fewer EPY (Vaclav et al. 2003). One possible explanation for such results is that, with increased food availability, females may range less widely, providing extra-pair males with fewer opportunities to engage in EPCs (Vaclav et al. 2003). Alternatively, supplemented females may perceive their social mates as being of higher quality because of the increased food availability and, therefore, be less likely to seek EPCs (O'Brien and Dawson 2011). In contrast to these studies, female European Serins (*Serinus serinus*) provided with supplemental food had more EPY in their broods than non-supplemented females (Hoi-Leitner et al. 1999), with supplemented females better able to circumvent the mate-guarding efforts of their social mates.

Mate guarding by males may limit the EPC opportunities for their social mate or the opportunities for conspecific males to seek EPCs with their social mates (Moller and Birkhead 1991, Fedy et al. 2002). Mate guarding is not always effective in protecting a male's paternity (Gowaty and Bridges 1991, Johnsen et al. 1998), but males provided with supplemental food may be able to spend less time foraging and more time mate guarding, potentially reducing their likelihood of being cuckolded. To date, however, the possible effect of food availability on the mate guarding behavior of male birds has not been tested experimentally.

Indigo Buntings (*Passerina cyanea*; hereafter buntings) are common, sexually-dichromatic birds that breed throughout the eastern United States (Payne 2006). Although socially monogamous, EPCs are a major component of bunting reproductive biology (Westneat 1988). For example, Westneat (1990) found that 35% of bunting nestlings resulted from extra-pair fertilizations (EPFs) in a population in North Carolina. Factors that influence the likelihood of female buntings engaging in EPCs are unclear. However, Carey and Nolan (1979) suggested that, when food is abundant, female buntings may be more likely to actively seek extra-pair partners.

Food availability can influence the breeding behavior of birds. However, based on a limited number of studies, variation in food availability may either increase or decrease the likelihood of females engaging in EPCs. In addition, the possible effect of food availability on male mate guarding behavior remains to be determined by experimental means. The objectives of my study were to use behavioral observations and genetic paternity analysis to determine how the presence of supplemental food might influence the behavior (singing, foraging, and mate guarding), aggression, and paternity status of male buntings. Based on the results of previous studies I predicted that, compared to non-supplemented males, supplemented males would: 1) spend less time foraging, 2) spend more time singing, 3) spend more time mate guarding, 4) respond more aggressively to a simulated intruder (i.e. playback of conspecific songs in their territories), and 5) have fewer EPY in their nests.

CHAPTER II

METHODS

Field work was conducted from 8 May – 15 August 2009 at the Miller-Welch/Central Kentucky Wildlife Management Area (CKWMA) in Madison County, Kentucky. The CKWMA encompasses 747 hectares and is dominated by shrubland with woodland fragments and narrow rows of shrubs and trees that provide suitable habitat for breeding buntings. Breeding territory boundaries were determined by spot-mapping locations of singing males and male-male interactions.

Throughout my study, adult buntings were primarily captured by passive netting. Mist-nets (6 or 9 m) were placed in territories where focal males were observed flying at low heights, or within 15 m of favored song perches. Males not captured by passive netting were captured by placing a speaker below a mist-net and playing conspecific songs to lure them into the net. Females were captured by placing 1 to 3 mist-nets near or around nests (within 5 m) with nestlings. Females were targeted for capture during the nestling stage to maximize capture success and minimize the likelihood of nest abandonment (Nisbit 1981, Safina and Burger 1983). Each adult bunting was banded with a numbered United States Geological Survey aluminum band and a unique combination of three colored-plastic bands to allow individual recognition.

Bunting nests were located by noting behavioral cues such as females carrying nesting material, food, or fecal sacs, and by searching likely nest sites. Once located, nests were visited every 2 – 3 days. Nestlings were banded and bled on days 5 or 6 post-

hatching to minimize the risk of premature fledging (young typically fledge 9 to 12 days post-hatching; Payne 2006). For nests found with nestlings, hatching dates were estimated based on a nestling period of 9 days (Taber and Johnston 1968, Payne 1982, Westneat 1988).

Supplementary food

The territories of eight male buntings ($n = 8$) in the study area were randomly selected for food supplementation, with an additional 22 territories and resident males serving as controls. Subsequently, I determined that males in seven of the 22 control territories used feeders in adjacent territories. Feeding stations consisted of hanging feeders (~5 m above ground to prevent use by White-tailed Deer [*Odocoileus virginianus*]) stocked *ad libitum* with millet. Feeders were placed in territories from 15 – 17 May to 15 August 2009 and were placed at the approximate center of bunting territories to minimize use by conspecifics in neighboring territories. The territory center was established with reasonable certainty by noting the location of song perches used by males. To ensure that males did not select territories based on the presence of supplementary food (Berthouly et al. 2008), feeders were set-up after territories had been established and prior to the earliest clutch completion. To determine which males used feeders, I noted the identity of uniquely banded individuals (see above for banding methods) visiting feeders during time-budget observations and also by videotaping feeders using camcorders (Sony, Model CCD-TRV138, Tokyo, Japan). Feeders were videotaped from 23 June to 1 August for a total of 194.7 hours (24.3 ± 1.0 hours/feeder).

Time budget activities

To determine time budgets, I observed male buntings (20 min/day of continuous observation). Time budget observations were conducted during the fertile period of the focal males' mate (see below for definition), because mate guarding behavior (to protect paternity) is contingent on the female being fertile. If an observation was performed outside of the fertile period, it was excluded from the analyses. Because the fertile period was difficult to identify in the field before a nest was found, I conducted observations every second or third day to make sure I had several repeated measurements during each female's fertile period. However, when a nest was not found in a territory, I usually conducted observations every fourth or fifth day. Each observation started when the focal male was first observed. During observations, I verbally described and recorded on tape (Sony TCM-59V Cassette Player/Recorder) all male behaviors including singing, foraging (with foraging defined as searching for natural food items), feeding at a feeder, mate guarding, perching, uttering 'chip' or 'tink' calls, and inter-male aggression. 'Chip' and 'tink' calls are both short duration (<0.2 sec) calls that extend over a wide range of frequencies, with 'chip' calls indicating mild distress or alarm and 'tink' calls indicating higher-intensity distress (Taber and Johnston 1968, Payne 2006). Because they are similar in duration and frequency range, 'chip' and 'tink' calls can be difficult to differentiate in the field and, as a result, I referred to all short-duration calls heard in the field as 'chip' calls.

On rare occasions when a male was hidden from my view in dense vegetation and away from his social mate, I assumed the male was foraging and not mate guarding. During these observations, I also recorded all songs on tape. An effort was made to

ensure that my presence and the running account of male behavior did not influence behavior or interfere with the recording of male song. Also, because singing rates vary with time of day (Taber and Johnston 1968, Thompson 1972, McNamara et al. 1987, Staicer et al. 1996, Liu and Kroodsma 2007), I made sure that time budget observations were not temporally biased toward either control or treatment males (mean observation time of 10:10 EST \pm 15.5 [SD] min, n = 40 for supplemented males and 10:35 EST \pm 16.9 min, n = 31 for non-supplemented males). Singing time was defined as the period starting with the first song delivered after at least 1 min of non-singing and ending with the last song delivered before not singing for at least 1 min. During female fertile periods, a male was considered to be mate guarding when within 5 m of his social mate or, if more than 5 m away, his gaze was directed toward his social mate. The fertile period was defined as a seven-day period, starting five days before the laying of the first egg to the laying of the penultimate egg (Moller 1985).

Song playback experiments

From 10 June – 27 July 2009, playback experiments were conducted with male buntings (N = 12) to quantify possible differences in male aggression between food-supplemented males (n = 6) and non-supplemented males (n = 6). To control for mating status (Kroodsma 1986), all playback experiments were performed when the mates of focal males were incubating full clutches. I only conducted playback experiments when the focal male's mate was incubating so that the presence of a mate did not influence male behavior. Upon entering a focal male's territory, I noted where the male started singing. All males included in playback experiments started singing within ~5 min of

territory entry. I then placed a speaker (Sony TCM-59V Cassette Player/Recorder) halfway between the male's nest (and incubating female) and the song perch and began playback of prerecorded, conspecific songs. An effort was made to ensure the volume was comparable to a typical bunting song. I then moved to a location within the territory where my presence would not influence male behavior, but where I could still observe the focal male. Distance from the speaker was typically about 30 m (range of 25 – 40 m) and always approximately equidistant to both the active nest and the initial song perch.

Playback experiments had three, 3-min periods: pre-playback, playback, and post-playback. During each period, I noted the estimated distance of the focal male from the speaker every 15 sec to calculate the mean distance from the speaker and also the closest approach to the speaker. I also recorded the number of songs, 'chip' notes, growl calls, flights (flying from one perch to another), fly-bys (within 1 – 2 m of speaker), and attacks (<1 m of speaker) for each period. I defined the growl call as a harsh, low-frequency call used in aggressive contexts (Morton 1977); this call is also referred to as the 'adult aaaa' call and is given when a bunting is threatening another bunting (Payne 2006). In addition, I recorded the amount of time it took for each male to reach the distance of closest approach to the speaker during the playback period and the amount of time each male remained at the closest distance to the speaker during the post-playback period.

Recordings of buntings used in the playback experiments were made on 6 and 7 June 2009 in Madison County, Kentucky. Bunting songs were recorded using a recorder (TCM-50DV, Sony, Tokyo, Japan) and directional microphone (ME-88, Sennheiser, Old Lyme, CT). All recordings were made at locations >1 km away from my study area to ensure recorded songs had not been heard previously by focal males. To avoid

pseudoreplication, no bunting song recording was reused on a male of the same treatment type (Kroodsma 1986).

Genetic analysis

After capture in mist-nets, a small blood sample (50-75 μ l) was obtained from the brachial vein of each bunting. Blood samples were collected from 30 males, 14 females, and 50 nestlings (from 19 broods). Blood was collected in microhematocrit capillary tubes and placed in 1.5-mL vials containing 200 μ l of Queen's lysis buffer (Seutin et al. 1991). Vials were then placed on ice in the field and later stored in a -20°C freezer.

The Chelex[®] 100 extraction method was used to extract DNA from blood (Walsh et al. 1991). Vials containing 1 μ l of blood and 200 μ l of Chelex[®] 100 were incubated at 56°C for 20 min in a dry bath incubator and then agitated for 10 sec. Vials were then incubated at 100°C for 10 min and then agitated again for 10 sec. Vials were then placed in a 10,000 rpm centrifuge for 3 min. Extracted DNA was then stored in a 4°C refrigerator until needed for polymerase chain reaction (PCR) to amplify the DNA. For each vial prepared for PCR, the mixture contained 15.4 μ l of DNA-free water, 2.2 μ l of ThermoPol buffer, 1.8 μ l each of forward and reverse primer, 0.9 μ l of dNTPs, 0.1 μ l Taq DNA polymerase, and 2 μ l of extracted DNA. Samples were then loaded into a thermal cycler machine (Eppendorf Mastercycler) set on a cycle of 95°C for 5 min, then a cycle repeated for 34 times: 95°C for 45 sec, 55°C for 45 sec, and 72°C for 1 min. The cycle ended with 72°C for 7 min.

Once the PCR process was completed, 5 μ l of PAGE loading dye was added to each sample. Amplified samples and a 10 or 25 base pair ladder (Invitrogen, Carlsbad,

CA) needed to size the amplified DNA fragments were run through a 4% polyacrylamide gel on an electrophoresis machine (Gibco BRL Sequencing System Model S2, Life Technologies, Invitrogen, Carlsbad, CA).

Five microsatellite loci were chosen from a set of 10 loci that specifically amplified bunting DNA (>15 alleles), including Ase 18, Pdo 3, Emb 27, Ctc 16, and LOX 1 (Table 1). To date, no published studies using microsatellites have been performed on buntings, so these loci had not been previously used for this species. One of the five loci (Pdo 3) had a high frequency of null alleles, so results generated with this locus were heeded with caution since it occasionally produced inconsistent results.

Paternity assignment

Gels used in determining paternity were developed using a modified protocol of the silver-staining method (Bassam et al. 1991). To briefly outline the procedure, gels were initially placed in a fix solution (10% ethanol, 0.5% acetic acid) for 7 min. Then gels were placed into a silver nitrate solution (0.1% AgNO₃) for 11 min. Gels were then quickly rinsed with distilled water and placed immediately into the developer solution (1.5% NaOH) until bands appeared. To stop any further developing, gels were placed back into the fix solution for 3 min. Finally, the gels were again rinsed with water and allowed to air dry.

Gels were scored by hand and scanned into a Macintosh computer for later comparison. All potential offspring of a pair of bunting adults were run on the same gel. Offspring were determined to be EPY if they mismatched the presumed father's genotype at three or more of the five loci used. Paternity analysis was performed using CERVUS

2.0, a program that uses co-dominant loci to determine parentage (Marshall et al. 1998). Across all five loci, the total exclusionary power of detecting EPY was 0.99 at a 95% confidence interval.

Table 1. Five microsatellite loci used in paternity analysis, sequences, number of alleles, observed heterozygosity, and the probability of excluding an unrelated offspring assuming the first parent's genotype is known, from Indigo Buntings at Miller-Welch/Central Kentucky Wildlife Management Area in Madison County, Kentucky.

Locus	Sequence (5' → 3')	Number of alleles	Observed heterozygosity	Probability of exclusion
LOX 1 ^a	(F) ATGATGGTAAGTCTAATGAAAGC (R) CCACACACATTCACTCTATTG	40	0.926	0.859
Pdo 3 ^b	(F) CTGTTCAATTAACACAGGT (R) AGTGAAACTTTAATCAGTTG	31	0.735	0.813
Ase 18 ^c	(F) ATCCAGTCTTCGCAAAAGCC (R) TGCCCCAGAGGGAAGAAG	27	0.853	0.764
CtC 16 ^d	(F) GGCTTACCTTGTTCCAC (R) GTTGGGGTTCCTGTCTTG	27	0.879	0.813
Emb 27 ^e	(F) TCCCCATGATGGTCTGTACC (R) GCTGACTGCTTGGCTGGAC	20	0.853	0.692

Sources: ^aPiertney, S. B., A. D. C. MacColl, P. J. Bacon, and J. F. Dallas. 1998. Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology* 7:1645-1654.

^bNeumann, K. and J. H. Wetton. 1996. Highly polymorphic microsatellites in the House Sparrow *Passer domesticus*. *Molecular Ecology* 5:307-309.

^cRichardson, D. S., F. L. Jury, D. A. Dawson, P. Salgueiro, J. Komdeur, and T. Burke. 2000. Fifty Seychelles Warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in *Sylviidae* species and their cross-species amplification in other passerine birds. *Molecular Ecology* 9:2225-2230.

^dTarvin, K. A. 2006. Polymorphic microsatellite loci from the American Goldfinch (*Carduelis tristis*) and their cross-amplification in a variety of passerine species. *Molecular Ecology* 6:470-472.

^eMayer, C., K. Schiegg, and G. Pasinelli. 2007. Isolation, characterization and multiplex genotyping of 11 autosomal and four sex-linked microsatellite loci in the reed bunting, *Emberiza schoeniclus* (*Emberizidae*, Aves). *Molecular Ecology Notes* 8:332-334.

Statistical analyses

For all time budget behaviors, I used repeated measures analysis of variance (ANOVA; SAS Institute 1989). The repeated measures procedure was used because multiple measurements were made for each male. ANOVA was also used to examine the response of male buntings to playback of conspecific songs. Tukey's post-hoc tests were performed to determine which stage was significantly different from other stages. Due to my small paternity data sample size, a Wilcoxon test was used to determine if the mean proportion of EPY/brood differed between supplemented and non-supplemented males. One-tailed tests were used throughout because I made specific *a priori* predictions. Means are reported \pm standard error (SE), and results of $P \leq 0.05$ were considered statistically significant.

CHAPTER III

RESULTS

I conducted a total of 71 time budget observations on 30 male buntings, including eight with feeders and 22 without feeders in their territories. Each male was observed for an average of 47.3 ± 5.7 min (range = 20 – 120 min). Direct observations, in addition to a review of videos made at feeders, revealed that seven of 22 males without feeders in their territories visited feeders in nearby territories and, therefore, had access to supplemental food. Because the behavior of males that visited feeders in the territories of other males may or may not have been influenced by access to supplemental food, I analyzed time-budget data in two different ways. I compared the behavior of males with feeders in their territories ($n = 8$) to males without feeders ($n = 22$), but, in addition, I also compared the behavior of all males that visited feeders (either in-territory or off-territory; $n = 15$) with males without feeders in their territories who, in addition, were not observed at feeders ($n = 15$).

Feeders ($n = 8$) were videotaped for a total of 194.7 hours (mean = 24.3 ± 1.0 [SE] hours/feeder). Focal males spent a total of 34.7 min (29 separate instances) at feeders. Review of videotapes revealed that 16 banded males, four unbanded males, five banded females, three unbanded females, and two banded fledglings used the feeders (Table 2). Nineteen buntings, including 12 banded males, five banded females, and two banded fledglings, visited only one feeder, either within their territory or visiting a feeder. Nine banded males (26 total occasions) and two banded females (one occasion each)

were observed at off-territory feeders, showing evidence that both male and female buntings were making extraterritorial forays. In fact, four buntings (all banded males) visited more than one feeder, with three males visiting two separate feeders and one male visiting three separate feeders.

Table 2. Status of all individuals that visited feeders.

Feeder number	Bunting number	Feeder status	Sex	Band status	Time at feeder (min)	Times at feeder
1	13	in territory	male	banded	14.82	16
2	11	in territory	male	banded	3.42	5
2	10	visitor	male	banded	4.15	7
2	12	visitor	male	banded	0.55	1
3	20	in territory	male	banded	18.58	20
3	B32	in territory	female	unbanded	1.65	1
3	19	visitor	male	banded	1.95	1
3	40	visitor	male	banded	0.92	1
3	62	visitor	male	unbanded	22.53	26
3, 4	38	visitor	male	banded	3.52/9.33	2/11
3, 4	42	visitor/in territory	male	banded	1.4/29.03	1/29
3, 5	21	visitor	male	banded	2.97/18.05	1/12
3, 7, 8	41	visitor	male	banded	1.63/21.78/2	2/22/1
4	B10	in territory	female	unbanded	3.97	5
5	22	in territory	male	banded	29.32	35
5	49	in territory	female	banded	20.58	15
5	55	visitor	female	banded	11.43	11
5	61	visitor	male	unbanded	3.47	3
6	27	in territory	male	banded	1.67	2
6	50	in territory	female	banded	19.37	13
6	B12	in territory	fledgling	banded	2.82	3
6	54	visitor	female	banded	2.52	2
6	unknown	visitor	male	unbanded	24.32	31
6	unknown	visitor	female	unbanded	4.58	3
6	G6	visitor	fledgling	banded	2.82	1
7	23	in territory	male	banded	26.02	28
7	24	in territory	female	banded	8.3	6
7	25	visitor	male	banded	2.78	2
7	58	visitor	male	unbanded	5.33	4
8	30	in territory	male	banded	11.52	9

Males with and without feeders in their territories

Male buntings with feeders in their territories ($n = 8$) spent significantly less time foraging (means = 8.6% vs. 23.8%; $F_{1,28} = 4.5$, $P = 0.044$) and significantly more time uttering ‘chip’ calls (means = 17.3% vs. 4.5%; $F_{1,28} = 9.4$, $P = 0.0049$) than males without feeders in their territories ($n = 22$; Figure 1). However, I found no significant difference between males with and without feeders in their territories in mean time spent singing ($F_{1,28} = 0.1$, $P = 0.82$), mate guarding ($F_{1,28} = 1.3$, $P = 0.26$), perching ($F_{1,28} = 0.1$, $P = 0.85$), fighting ($F_{1,28} = 0.7$, $P = 0.41$), or at feeders ($F_{1,28} = 1.7$, $P = 0.20$; Figure 1).

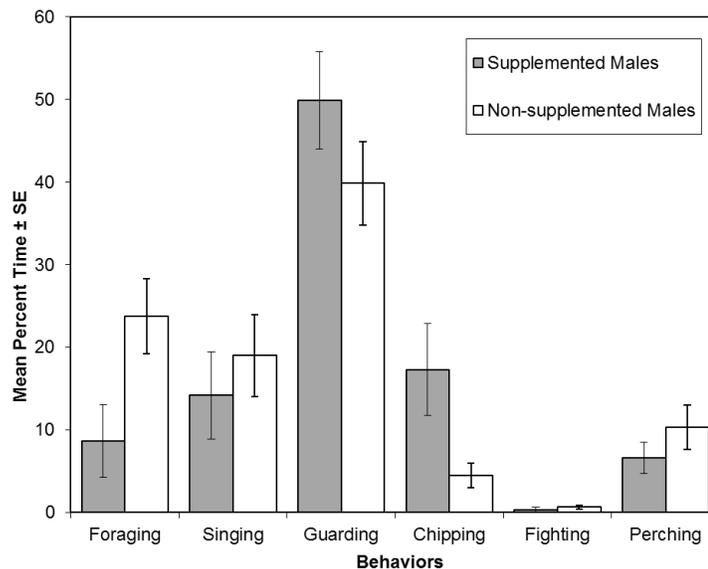


Figure 1. Effect of supplemental food on the behaviors of male Indigo Buntings with ($n = 8$) and without feeders ($n = 22$) in their territories (mean time spent foraging, singing, mate guarding, chipping, fighting, and perching).

Males observed at feeders vs. males not observed at feeders

Male buntings with feeders in their territories and males without feeders in their territories, but observed at feeders ($n = 15$), spent significantly less time foraging (means = 10.6% vs. 28.8%; $F_{1,28} = 8.3$, $P = 0.0077$) than male buntings not observed at feeders (n

= 15; Figure 2). However, I found no significant difference between males observed and not observed at feeders in time spent in other activities, including singing ($F_{1,28} = 0.6$, $P = 0.46$), chipping ($F_{1,28} = 2.9$, $P = 0.10$), mate guarding ($F_{1,28} = 2.5$, $P = 0.13$), perching ($F_{1,28} = 0.1$, $P = 0.97$), and fighting ($F_{1,28} = 1.7$, $P = 0.20$; Figure 2).

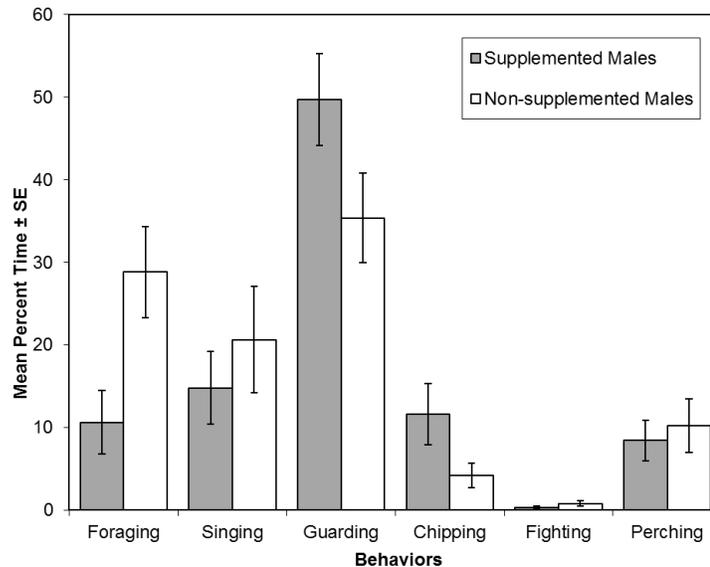


Figure 2. Effect of supplemental food on the behaviors of male Indigo Buntings observed at ($n = 15$) and not observed at feeders ($n = 15$; mean time spent foraging, singing, mate guarding, chipping, fighting, and perching).

Response to playback of conspecific songs

Overall (food supplemented and non-supplemented males combined), male buntings responded to playback of conspecific songs in their territories by moving closer to ($F_{1,10} = 21.0$, $P < 0.0001$) and remaining close ($F_{1,10} = 13.0$, $P < 0.0001$) to the speaker, uttering more ‘chip’ calls ($F_{1,10} = 4.0$, $P = 0.028$) and growl calls ($F_{1,10} = 3.6$, $P = 0.039$), and making more flights ($F_{1,10} = 13.1$, $P < 0.0001$). During playback, male buntings did not utter more songs ($F_{1,10} = 1.2$, $P = 0.31$) or make more fly-bys ($F_{1,10} = 2.2$, $P = 0.13$) than before (pre-playback) or after (post-playback) playback.

Comparison of the responses of males with ($n = 5$) and without feeders ($n = 7$) in their territories to playback revealed no differences in the closest distance approached ($F_{1,10} = 1.2$, $P = 0.31$), number of songs ($F_{1,10} = 0.1$, $P = 0.73$), chipping ($F_{1,10} = 0.1$, $P = 0.84$), or growl calls ($F_{1,10} = 0.1$, $P = 0.73$) uttered, or number of fly-bys ($F_{1,10} = 1.7$, $P = 0.23$). However, differences in the mean distance from the speaker during playback ($F_{1,10} = 3.6$, $P = 0.088$), number of flights ($F_{1,10} = 4.6$, $P = 0.057$), and time needed for males to reach their closest approach distance ($F_{1,10} = 3.7$, $P = 0.077$) approached significance, with males with feeders in their territories maintaining a closer mean distance from the speaker, making more flights, and reaching their closest distance from the speaker sooner than males without feeders (Table 3). In addition, after playback ended, males without feeders in their territories remained at their closest approach distance significantly longer than did males with feeders in their territories ($F_{1,10} = 10.0$, $P = 0.007$; Table 3).

Table 3. Comparison of mean responses of male Indigo Buntings with ($n = 5$) and without feeders ($n = 7$) in their territories to playback of conspecific song.

Variable ¹	Supplemented males	Non-supplemented males
Distance from speaker	11.1 ± 0.4 m	16.6 ± 2.4 m
Number of flights	5 ± 1	3 ± 1
Time to reach closest approach	54 ± 17 sec	69 ± 15 sec
Time remained at closest approach	36 ± 11 sec	96 ± 25 sec

Note: ¹closest approach = distance of closest approach to the playback speaker

Clutch sizes, brood sizes, and extra-pair paternity

Due to a high rate of nest predation (30 of 43 nests located, or 69.8%, were predated prior to fledging), I was only able to determine the paternity status at 16 nests

(blood samples of nestlings in some nests were obtained before nests were predated so some nests where I examined paternity did not fledge any young). For those nests where I examined paternity, the mean clutch size was 2.9 ± 0.2 ($n = 11$) and the mean brood size was 2.7 ± 0.2 ($n = 16$). Of 16 broods, 12 (75%) had at least one EPY and, in those broods, 24 of 43 nestlings (56%) were EPY. The difference in proportion of EPY in nests in territories with feeders (mean = 0.8 ± 0.2 , $n = 8$) and territories without feeders (mean = 0.4 ± 0.1 , $n = 8$) approached significance ($z = 1.8$, $P = 0.079$; Figure 3).

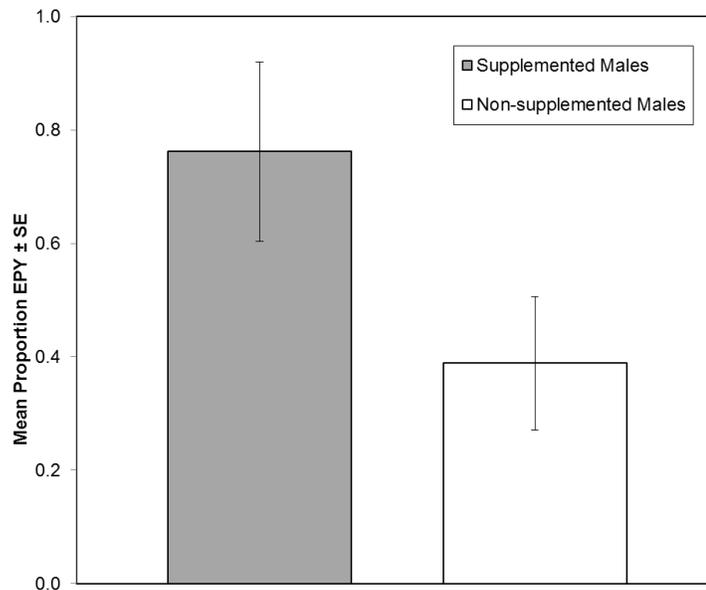


Figure 3. Effect of supplemental food on male Indigo Bunting mean proportion of extra-pair young in nests on territories with ($n = 8$) and without feeders ($n = 22$).

CHAPTER IV

DISCUSSION

I found that food-supplemented male buntings spent less time foraging for natural food items than non-supplemented males. Other investigators have also reported that male songbirds provided with supplemental food spent less time foraging. For example, Tobias (1997) found that food-supplemented male European Robins (*Erithacus rubecula*) allocated less time to foraging (~45%) than non-supplemented males (~60%). Similarly, male Dunnocks (*Prunella modularis*) that visited feeding stations spent less time foraging (32.9%) than control males (72.6%) (Davies and Lundberg 1984). Cucco and Malacarne (1997) found that food-supplemented male Black Redstarts spent approximately 10% of their time foraging compared to approximately 58% of the time for non-supplemented males.

Davies and Lundberg (1984) provided Dunnocks with supplemental food and found that, compared to controls, food-supplemented males, with less time foraging, spent more time perched (47.8% vs. 17.5%) and interacting (16.1% vs. 7.0%), and sang at higher rates (126.4 songs/hour vs. 56.7 songs/hour). Similarly, food-supplemented Black Redstarts also spent more time being vigilant (~35% vs. ~20%) and more time singing (~25% vs. ~5%) than control males (Cucco and Malacarne 1997).

Male buntings that visited feeders in my study spent more time chipping (11.6%) than males that did not visit feeders (4.2%). Buntings utter 'chip' calls (and similar-sounding 'tink' calls) in a variety of contexts and these calls indicate varying levels of

distress (Payne 2006). For example, male buntings utter these calls when conspecific males enter their territories (Payne 2006). During my playback experiments, male buntings uttered significantly more ‘chip’ calls during playback (i.e., a simulated intrusion) and post-playback periods than during the pre-playback period. Thus, one possible explanation for the greater time spent uttering ‘chip’ calls by food-supplemented males in my study was an increase in the frequency of intrusion into their territories by conspecific males attempting to access the feeders. Conspecific males were observed (and video-recorded) at feeders and so, although rarely observed during focal-male observations, such trespassing into the territories of food-supplemented males did occur. Other investigators have also noted varying degrees of aggression by territorial males in response to trespassing conspecific males (Searcy 1979, Martin 1987, Draycott et al. 2005, Brown and Sherry 2006, Robb et al. 2008).

In addition to ‘chip’ and ‘tink’ calls, male songbirds sing to establish, maintain, and defend territories (Nowicki and Searcy 2004, Searcy et al. 2006, Robb et al. 2008). Some investigators have found that males provided with supplemental food sing at higher rates than non-supplemented males, including male Australian Reed Warblers (*Acrocephalus australis*; Berg et al. 2005), male European Robins (Thomas 1999), and male Pied Flycatchers (Alatalo et al. 1990). In contrast, I found no difference between food-supplemented and non-supplemented male buntings either in time spent singing or the number of songs uttered during playback of conspecific songs in their territories. One possible explanation for the absence of any difference in time spent singing by supplemented and non-supplemented males is that I collected time-budget data during female fertile periods when male buntings tend to sing less frequently (Payne 2006).

During female fertile periods, I found that male buntings spent much of their time mate guarding, remaining close to females. Payne (2006) reported a similar behavior. When mate guarding, male buntings spend much of their time lower in the vegetation with females (pers. observ.). In contrast, when singing, male buntings typically choose higher perches (Thompson 1972, Hylton and Godard 2001, pers. observ.). Thus, males spending much of their time mate guarding tend to spend correspondingly less time singing from their typically high perches.

In addition, during song playback experiments, playback and post-playback periods combined lasted only 6 min and any effects of food supplementation on responses of male buntings during such a short period would likely be minimal. Similarly, Strain and Mumme (1988) found that food-supplemented and non-supplemented male Carolina Wrens uttered songs at similar rates in response to playback of conspecific songs in their territories.

Playback experiments

In response to playback of conspecific songs in their territories, male buntings in my study approached closer to the speaker, stayed closer to the speaker, uttered more ‘chip’ and growl calls, and made more flights than during the pre-playback period. Males in many other species of songbirds have also been found to respond in similar ways when conspecific songs are played back in their territories (Searcy et al. 2000, 2006, Olendorf et al. 2004, Ballentine et al. 2008, Hof and Podos 2013).

Although differences only approached significance, food-supplemented male buntings in my study responded to playback by making more flights, approaching the

speaker more rapidly, and maintaining a closer mean distance from the speaker than non-supplemented males. Food-supplemented male buntings may tend to respond more aggressively to playback than non-supplemented males because they may perceive their food-supplemented territories as being higher in quality and, therefore, are more willing to respond aggressively in territory defense. For example, Golabek et al. (2012) found that groups of food-supplemented Pied Babblers (*Turdoides bicolor*) responded to simulated territorial intrusion (song playback) faster than, and for longer periods than, non-supplemented groups. These authors suggested that differences in the responses of food-supplemented and non-supplemented groups may have been due to energetic constraints, with birds in non-food-supplemented groups more energetically constrained and, therefore, less able to respond to the simulated intrusion. Similarly, Ydenberg (1984) reported that food-supplemented male Great Tits (*Parus major*) responded more vigorously to simulated territory intrusions (playback of conspecific songs along with presentation of a taxidermy mount of a male Great Tit) than non-supplemented males, and suggested that food-supplemented males were able to spend less time foraging and were also able to invest more time and energy toward territorial defense.

During the post-playback period, non-food-supplemented male buntings in my study remained at their closest approach distance significantly longer than food-supplemented males. One possible explanation for this is that rates of territorial intrusions by conspecific males may have been higher in the food-supplemented territories. Indirect evidence of this comes from the observation of neighboring male buntings visiting feeders in those territories and from the high rates of chipping by food-supplemented

males. If so, then food-supplemented males may have left the vicinity of the speakers sooner than non-supplemented males to continue guarding their fertile mates.

Mate guarding and extra-pair paternity

During female fertile periods, male buntings in my study, both those with (49.9% of their time) and without (39.9% of their time) feeders in their territories, spent more time mate guarding than engaged in any other activity. Despite this, 12 of 16 broods (75%) had at least one EPY and 24 of 43 nestlings (56%) were EPY. In addition, although the difference only approached significance ($P = 0.079$), the proportion of EPY in food-supplemented territories (mean = 0.8) was higher than in non-food-supplemented territories (mean = 0.4). Similarly, European Serins in territories with greater food abundance also had more EPY (Hoi-Leitner et al. 1999). In contrast, male Red-winged Blackbirds (Westneat 1994), House Sparrows (Vaclav et al. 2003), and Mountain Bluebirds (O'Brien and Dawson 2011) provided with supplemental food had fewer EPY in their nests than non-supplemented males.

One possible explanation for the presence of more EPY in the nests of food-supplemented male buntings in my study is that feeders attracted neighboring males and provided females in those territories with more opportunities to engage in EPCs. In addition, based on the constrained female hypothesis (Gowaty 1996), females in non-food-supplemented territories may be more energetically and time limited (i.e., need to spend more time foraging than females in food-supplemented territories) and, therefore, have less time to seek EPCs in neighboring territories. In support of that hypothesis, Humbird and Neudorf (2008) found that female Northern Cardinals (*Cardinalis*

cardinalis) provided with supplemental food made more extra-territorial forays and spent more time off territory than non-supplemented females.

My results, with more EPY in food-supplemented territories, also suggest that female buntings do not use the presence of supplemental food as an indicator of habitat or male quality. If they did, neighboring, non-supplemented females might be expected to pursue EPCs with food-supplemented males (i.e., more EPY in non-supplemented territories), and food-supplemented females would not pursue EPCs (i.e., fewer EPY in supplemented territories).

Mate guarding can also influence paternity. However, both food-supplemented and non-supplemented male buntings in my study spent more time mate guarding during their mates' fertile periods than engaged in any other activity and rates of EPP were still higher than reported in most previous studies of extra-pair behavior in songbirds (Wan et al. 2013). Such results do not necessarily suggest that mate guarding by male buntings is ineffective, because rates of EPP might be even higher if males spent less time mate guarding. For example, male Northern Wheatears (*Oenanthe oenanthe*; Currie et al. 1999), Black-throated Blue Warblers (*Setophaga caerulescens*; Chuang-Dobbs et al. 2001a), and House Wrens (*Troglodytes aedon*; Brylawski and Whittingham 2004) that were captured and temporarily detained were cuckolded at higher rates than control males. In addition, Wagner et al. (1996) and Marthinsen et al. (2005) found that male Purple Martins (*Progne subis*) and male Reed Buntings (*Emberiza schoeniclus*) that guarded mates more intensely and for longer periods had significantly fewer EPY in their nests.

My results do suggest that mate guarding by male buntings did not constrain females from engaging in EPCs. One proximate explanation for the high rates of EPP in buntings is that their early successional habitats have scattered areas of dense vegetation that likely allow females to elude mate-guarding males. Other investigators have also suggested that female songbirds may be better able to make extra-territorial movements or engage in copulations with trespassing males in habitats with reduced visibility (Mays and Ritchison 2004, Blomqvist et al. 2005). In addition, although male buntings spent much time mate guarding, even food-supplemented males only spent about half of their time guarding their mates during their fertile periods. Thus, female buntings would likely have ample opportunities to seek EPCs.

What is less clear is why rates of EPP are relatively high in buntings (Westneat 1987, 1990, this study; Figure 4) relative to most other songbirds that have been studied (Wan et al. 2013).

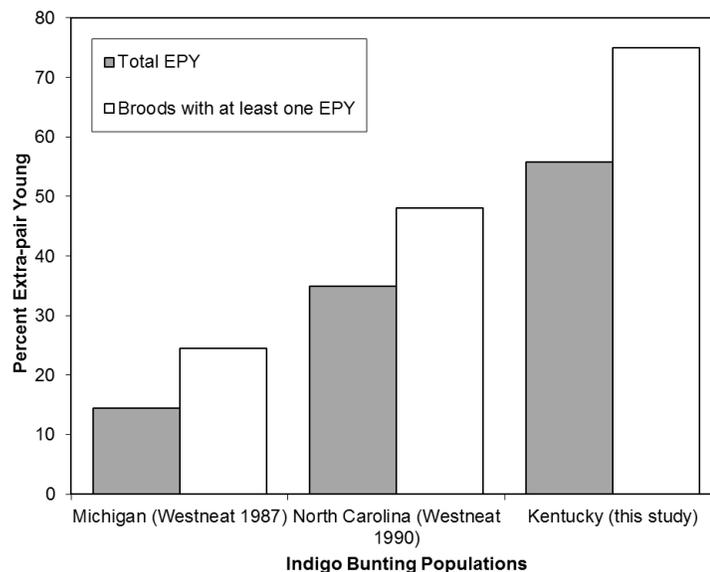


Figure 4. Comparing male extra-pair paternity data for three different Indigo Bunting populations: Cass County, Michigan (Westneat 1987), Orange County, North Carolina (Westneat 1990), and Madison County, Kentucky (this study).

The high rates of EPP in food-supplemented territories suggest that females are likely not engaging in EPCs for a direct benefit such as access to food as reported in some other species of songbirds (e.g., Red-winged Blackbirds; Gray 1997). In addition, female buntings do not risk the loss of male assistance in provisioning nestlings by engaging in EPCs, as reported in some species (Dixon et al. 1994, Chuang-Dobbs et al. 2001b), because male buntings rarely aid their mates in provisioning nestlings (Ritchison and Little 2014).

Additional study is needed not only to better understand the high rates of EPP in buntings, but the high rates of EPP in other species of songbirds as well. Factors that might contribute to high rates of EPP in buntings and other songbirds include increased genetic quality of their offspring, e.g., better genes (additive effect) or more compatible genes (non-additive effect). Mating preferences for mates with compatible genes could maximize offspring heterozygosity, minimize the risks of close inbreeding, or optimize immunogenetic complementarity (Schmoll 2011). Gohli et al. (2013) found that species of songbirds with higher rates of EPP had major histocompatibility complex (MHC) alleles with greater sequence diversity. Importantly, these MHC alleles encode antigen-presenting molecules that are important in avian immune responses (Klein 1986), and greater diversity in these alleles should allow immune systems to recognize a wider variety of pathogens (Gohli et al. 2013).

In sum, food-supplemented male buntings spent less time foraging and more time vocalizing ('chip' calls) than non-supplemented males. However, the potential benefits of spending less time foraging and being engaged in other activities may have been counteracted by the presence of trespassing males; males potentially seeking access to

supplemental food and to copulate with the mate of food-supplemented males. Regardless of the cause, food-supplemented males tended to be cuckolded at higher rates than non-supplemented males. The extent to which this was due to the presence of supplemental food attracting neighboring males, as well as the extent to which food-supplemented males may have been able to benefit from the additional food by having more time and energy to engage in EPCs with neighboring females (and thus negate, at least to some extent, the increased number of EPY in their own nests) are unclear.

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