

RESEARCH PAPERS

Egg Coloration and Recognition of Conspecific Brood Parasitism in Eastern Bluebirds

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Abstract

Individual eastern bluebird (*Sialia sialis*) females produce clutches of eggs with unique coloration and older females and females in better body condition lay more pigmented blue-green eggs. Conspecific brood parasitism in this species is not uncommon and bluebirds occasionally reject what appear to be normal eggs by moving them to the periphery of the nest. I used UV-visual reflectance spectrometry to objectively measure coloration of eggs and nest material. To estimate the conspicuousness of the trait, I calculated the contrast between eggs and background nest material. I found high achromatic and chromatic contrast between the coloration of eggs and of the nests, suggesting that bluebird eggs are highly conspicuous. To test the hypothesis that expression of blue-green coloration eggs facilitates recognition of eggs laid by conspecific brood parasites, I cross-fostered individual eggs into host nests during egg laying and monitored the fate of those eggs. I found no support, however, for the hypothesis that egg coloration facilitates discrimination of parasitic eggs from host eggs.

Introduction

Conspecific brood parasitism, whereby females lay eggs in the nests of conspecifics but provide no care for the offspring, is fairly common in birds (Eadie et al. 1998). Although egg recognition and subsequent rejection is a common defense in avian hosts parasitized by interspecific brood parasites, host defenses based on conspecific egg recognition are rarer (Davies 2000), presumably because either there is insufficient variation in egg features among conspecific females (Andersson 1984) or because parasitism does not pose enough of a fitness cost to hosts (Davies et al. 1996). Recent evidence from American coots (*Fulica americana*) suggests, however, that conspecific parasitic eggs that are rejected differ more in color from host eggs than do accepted parasitic eggs (Lyon 2003).

Mechanical assessments of blue-green egg coloration using ultraviolet-visible reflectance spectrometry

have recently shown that, in some species of passerines, individual females lay clutches of uniquely colored eggs (Moreno et al. 2004; Siefferman et al., 2006). The pigment biliverdin, which colors eggs blue and green (Kennedy & Veevers 1976), is deposited on the shell by the mother's shell gland. Biliverdin-based, blue-green egg coloration is correlated to female body condition, age (Siefferman et al., 2006) and immunocompetence (Moreno et al. 2005) in songbirds. This correlation may arise because biliverdin and its reduction product, bilirubin, function as antioxidants (McGraw 2005). The evolution of distinctive and condition-dependent egg coloration of individual females could facilitate discrimination of eggs from those laid by a brood parasite (Davies & Brooke 1988; Møller & Petrie 1991), particularly if females in lower condition are less likely to secure nesting cavities and are, therefore, more likely to parasitize the nests of high condition females.

Eastern bluebirds (*Sialia sialis*) are socially monogamous songbirds that experience relatively high levels of conspecific brood parasitism (up to 10% of offspring; Gowaty & Karlin 1984). Potential responses to brood parasitism include removing eggs from nests, covering clutches with new nest material (Gowaty & Plissner 1998), abandoning nests and moving eggs to the periphery of the nest (edge of the nest, resting against nest box) where they are not incubated (L. Siefferman, pers. obs.). Eastern bluebirds breed in cavities that they do not excavate themselves (secondary cavity nesters) and cavities are often limited (Gowaty & Plissner 1998). Experimental manipulations of nest-site availability indicate that female bluebirds that do not have their own nesting cavities adopt a strategy of parasitism (Gowaty & Bridges 1991). Female bluebirds display behaviors that likely protect maternity including cavity guarding (Gowaty et al. 1989) and engaging in aggressive interactions with intruding females (Gowaty & Wagner 1988).

Female bluebirds lay blue-green eggs that are colored by the pigment biliverdin (T. J. Robinson, unpubl. data). Female eastern bluebirds uniquely color their clutches, and older females and those in better body condition lay more pigmented eggs (Siefferman et al., 2006) and experience higher reproductive success than younger and poorer condition females (Siefferman & Hill 2005). In addition to typical interclutch variation in blue-green coloration of eggs, <3% clutches are white or pink (Siefferman et al., 2006) and color morphs are consistent within females (within clutches and among successive clutches; Gowaty & Plissner 1998).

To investigate whether bluebirds use egg coloration to detect brood-parasitic eggs, I cross-fostered individual eggs into host clutches and monitored nests for evidence of rejection. Moreover, because very conspicuous traits suggest a signaling function (Schluter & Price 1993), I estimated the conspicuousness of bluebird eggs in nests by assessing spectral contrasts of eggs and the background nest material.

Methods

I studied a wild population of eastern bluebirds from Apr. to Jun. 2005 in Lee County, AL, USA (32°35'N, 82°28'W). I visited nest boxes twice weekly at the beginning of the breeding season and when complete nests were found, I visited nest boxes daily to mark each egg. Because eastern bluebirds lay one egg daily between 700 and 1000 h (Meek & Robertson 1995), the arrival of two eggs on the same day

can indicate conspecific parasitism. I visually classified clutches of eggs as dark blue-green, pale blue-green, white, or pink and then objectively measured coloration using a reflectance spectrometer (see detailed methods below).

I monitored 38 nests for which there was no evidence of natural brood parasitism. For experimental nests, I paired nests with a common first egg date and nests in which clutches appeared different in coloration to the human observer. I cross-fostered one egg in each clutch within a 30 min time period between 1000 and 1200. I attempted to cross-foster eggs that were similar in size. Eggs were cross-fostered during the end of the egg-laying sequence (on the day that the fourth or fifth egg was laid) and prior to incubation. In most cases, I swapped eggs between the two nests such that clutch size remained unchanged. One female bluebird, however, was killed by a house sparrow (*Passer domesticus*) just after laying a clutch of pink eggs. Each of these four eggs were moved into four host nests that had blue-green eggs (clutch size = 4) and thus clutch sizes increased by one egg. I cross-fostered eggs from 18 nests (four of which experienced an increase in clutch size from four to five eggs). In one nest, I placed a white egg into a blue-green clutch and in one nest, I placed a blue-green egg into a white clutch. In 12 nests, I swapped blue-green eggs. These nests were compared to 20 control nests (mean brood size = 4.2) in which there was no evidence of brood parasitism. After completion of clutches, I monitored nests twice weekly to quantify egg removal, movement of eggs to the periphery of the nest and nest abandonment. I also quantified nestling mortality as the death or disappearance of a nesting prior to the expected fledging date (between day 16 and 19 post-hatch).

For all clutches, I measured reflectance with a portable spectrometer (range: 300–700 nm; S2000, Ocean Optics Inc., Dunedin, FL, USA), a Xenon pulse lamp and a fiber-optic probe. One day following the egg swap, I removed the eggs from the nest boxes and blocked the entrances of the nest boxes with cloth. Spectral measurements of eggs were taken from inside a vehicle within 25 m of the nest box and eggs were returned to the nest within 15 min. The measurements illuminated a region 2 mm in diameter and were taken at a 90-degree angle to the egg shell surface. Each reading was constructed from an average of 20 reflectance curves. I took three readings from each egg, moving the probe by at least 5 mm before taking each new reading. Blue-green chroma was calculated as the proportion

of the total reflectance that is in the blue-green region ($R_{400-575}/R_{300-700}$) of the spectrum. I used blue-green chroma to describe egg reflectance data because this region corresponds to the region of least absorbance (and therefore greatest reflectance) of biliverdin (Falchuk et al. 2002) and because typical bluebird eggs reflect light maximally in this region (Fig. 1). Additionally, for a subset of the clutches, I measured the reflectance of 10 bluebird nests (made of pine needles or dry grasses). Following the methods of Hunt et al. (2003), I calculated the normalized contrasts between the spectral reflectance of the nest background and the egg $[(R_{\text{egg}} - R_{\text{nest}})/R_{\text{egg}} + R_{\text{nest}}]$; Fig. 2].

I tested for normality using Shapiro–Wilk tests. Data were normally distributed and thus parametric tests were used. I used SPSS (version 11.5 Chicago, SPSS 2004) software to analyze the data and all statistical tests were two-tailed.

Results

Bluebirds laid eggs of three morphs: 36 nests were blue-green, one was white, and one was pink, and there was a discrete difference in spectral shape between blue-green, white and pink eggshells (Fig. 1). Using ANCOVA with nest of origin as the fixed factor and number of eggs measured per clutch as the covariate, I found a significant effect of nest

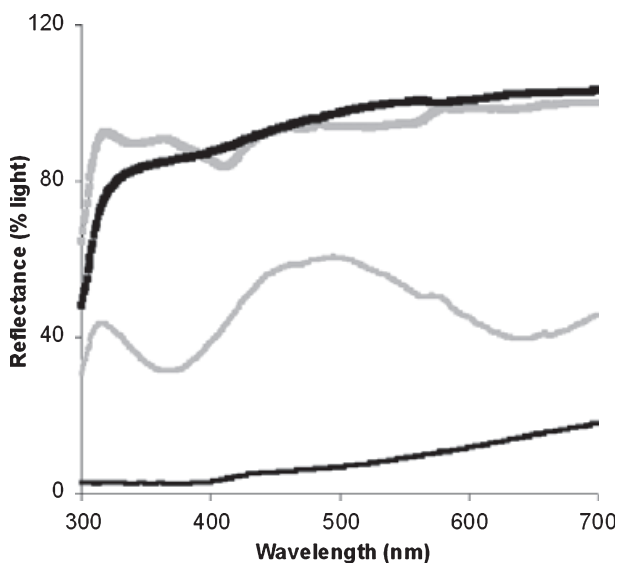


Fig. 1: Reflectance spectra of typical blue-green (thin grey line), pink (thick grey line), and white (thick black line) eggs of eastern bluebirds and reflectance spectra of nest material (thin black line) of eastern bluebirds

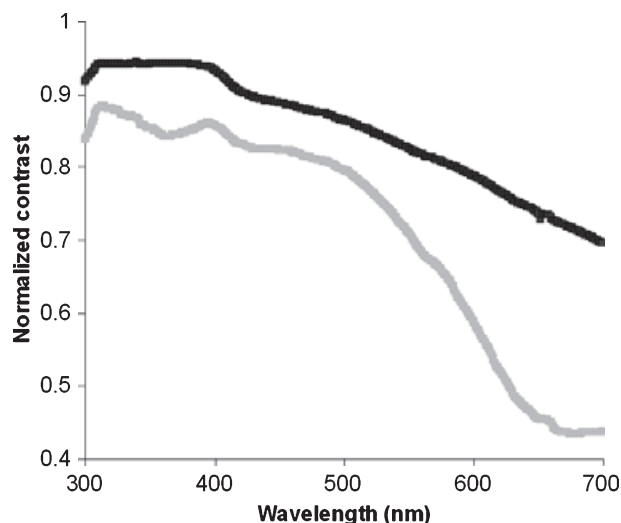


Fig. 2: Normalized contrasts (300–700 nm) between the spectral reflectance of nest background and eggs $(R_{\text{egg}} - R_{\text{nest}}/R_{\text{egg}} + R_{\text{nest}})$ of eastern bluebirds. The grey line is the contrast between the nest material and blue-green eggs, the black line is the spectral contrast between the nest material and white eggs (pink and white eggs were nearly identical in contrast)

of origin on egg coloration (ANOVA, $F_{38,153} = 10.4$, $p < 0.001$) prior to the cross fostering experiment, indicating that individual females laid eggs that differed from the eggs of other females. When I included only blue-green eggs, the effect of nest of origin remained significant (ANOVA, $F_{36,146} = 8.7$, $p < 0.001$).

I compared the blue-green chroma of every swapped egg with the host eggs by ranking the coloration (blue-green chroma). In all cases, the fostered egg was the most distinctive in each clutch, demonstrating that the experiment was successful in placing one distinctively colored egg in each host clutch. In 11 of 18 nests, the swapped egg exhibited the least blue-green chroma compared to all of the other eggs in the host clutch. In seven of 18 nests, the swapped egg exhibited greater blue-green chroma than all of the host eggs.

In two of 18 experimental clutches, one egg was moved to the nest periphery and in one of 18 nests, an egg disappeared during the incubation stage. However, none of the cross-fostered eggs were moved or removed from the nest. In control nests, one egg was moved to the periphery of the nest and one nest was abandoned. Nestling mortality occurred in none of the nests. I used Fisher's exact tests to compare rates of abandonment, egg movement, and removal and nestling mortality in experimental and control broods. Within experimental broods, I used

Fisher's exact tests to compare rates of egg movement or removal, and rates of nestling mortality in control and parasitic nests. There were no differences in the rates of removal, movement and abandonment of clutches, or nestling mortality between experimental and control broods (all $p > 0.70$). Moreover, in experimental nests, parasitic eggs were not more likely to be removed ($p = 0.76$) or moved ($p = 0.76$) or discriminated against (both removal and movement; $p = 0.76$) than host eggs. Finally, rates of egg removal, movement, nest abandonment and nestling mortality did not differ between nests in which hosts were given a parasitic egg of a different color morph (white, pink and blue-green) compared to host nests given parasitic eggs of the same color morph (all $p > 0.67$).

Discussion

I found no evidence that the blue-green coloration of the eggs of eastern bluebirds facilitates discrimination of host eggs from those laid by a brood parasite. Although cross-fostered eggs differed in coloration from the host clutch, they were not more likely to be moved to the periphery of the nest or removed from the nests. I found, however, evidence that supports a signaling function of blue-green coloration of the eggs of eastern bluebirds. I found high achromatic and chromatic contrast between bluebird eggs and the background nesting material, suggesting that bluebird eggs are probably highly conspicuous, even in the low light environment typical of a nest box (Hunt et al. 2003). All three egg color morphs reflected ultraviolet light, while the nests reflected very little short wavelength light, but reflectance increased gradually toward the longer wavelengths (Fig. 1). This resulted in a large contrast in reflection between the egg and the nests (Fig. 2), suggesting that eggs are conspicuous against the background.

Because maternal care is costly and should decrease the parental investment available for current and future offspring, selection against uncertain maternity is expected to result in behavior that protects genetic maternity (Trivers 1972). There is evidence that conspecific egg discrimination is well developed in coots and rails (Lyon 1993), ostriches (Bertram 1992) and weaverbirds (Jackson 1998). None of the bluebirds in this study rejected a parasitic egg, compared to American coots in which 43% of hosts reject parasitic eggs (Lyon 2003). Although the sample sizes in this experiment were relatively low, a third of the experimental nests included parasitic eggs of different color morphs (white, pink and

blue-green) than the host clutch and none of these eggs were discriminated against. Thus, it appears that eastern bluebirds do not use egg color variation alone to discriminate against conspecific brood parasitic eggs.

Any of the following reasons may explain why bluebirds do not discriminate against conspecific parasitic eggs: the cost of accepting parasitic eggs is low (Brooker & Brooker 1996), the cost of recognition is high (Davies et al. 1996), or rates of parasitism are low (Davies et al. 1996). Natural selection for recognition is probably stronger in species in which parasitism causes the death of host chicks (either directly or indirectly via competition for food resources). Conspecific brood parasitism in bluebirds is not accompanied by removal of host eggs (Gowaty & Plissner 1998) and thus parasitism increases the total number of chicks in host nests. However, unlike American coots in which success of the parasitic chick comes at the expense of a host chick (Lyon et al. 2002), parasitism in this population of eastern bluebirds may have no or very little effect on the host parents' or host siblings' fitness. Chick starvation is not common in this population; after excluding depredated nests, nestling mortality occurs in only 11.6% of nests (622 nests; L. Siefferman, unpubl. data). Thus, rearing brood parasitic eggs may not result in significant fitness loss. It is also possible that parasitism rates are low enough that bluebirds have not evolved a mechanism to detect parasitic eggs.

Hosts sometimes make recognition errors when faced with parasitic eggs and reject one of their own eggs rather than the foreign egg (Davies & Brooke 1988). Hosts should vary their response according to the risk of making mistakes, payoffs from rejection and the probability of parasitism (Rothstein 1982). Because of recognition errors, acceptance may be the best course of action when there are low probabilities of parasitism (Davies et al. 1996). Although the conspecific parasitism rate in this population is not known, levels of parasitism in other populations vary with year, availability of nest site and other ecological factors, and account for 1–10% of nestlings (Gowaty & Bridges 1991). Moreover, in this population, interspecific parasitism by brown-headed cowbirds (*Molothrus ater*) and other species is rare, with only 0.002% of nests parasitized (622 nests; L. Siefferman, unpubl. data).

Protection of maternity can take a variety of forms including egg discrimination, aggressive responses to intruding females and nest guarding (Andersson 1984). Female eastern bluebirds guard their boxes

during the egg laying period; they forage closer to the nest box when nests are most vulnerable to parasitism and this behavior is more pronounced when the threat of conspecific nest parasitism is greatest (Gowaty et al. 1989). Perhaps, for eastern bluebirds, nest guarding and aggression toward conspecific females are the primary mechanisms to avoid brood parasitism. An improved experimental design would present a conspecific female near the nest and simultaneously add an egg of unique coloration to the nest. Future research should also explore alternative functions for condition-dependent and conspicuous egg coloration in this species.

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Literature Cited

- Andersson, M. 1984: Brood parasitism within species. In: Producers and Scroungers: Strategies of Exploitation and Parasitism (Barnard, C. J., ed.). Croom Helm, London, pp. 195–227.
- Bertram, B. C. R. 1992: The Ostrich Communal Nesting System. Princeton Univ. Press, Princeton.
- Brooker, M. & Brooker, L. 1996: Acceptance of the splendid fairy-wren of parasitism by Horsfield's bonze cuckoo: further evidence of evolutionary equilibrium in brood parasitism. *Behav. Ecol.* **7**, 395–407.
- Davies, N. B. 2000: Cuckoos, Cowbirds, and Other Cheats. Poyser, London.
- Davies, N. B. & Brooke, M. de L. 1988: Cuckoos vs. reed warblers: adaptation and counter adaptations. *Anim. Behav.* **36**, 262–284.
- Davies, N. B., Brook, M. de L. & Kacelnik, A. 1996: Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proc. R. Soc. Lond. B Biol. Sci.* **263**, 925–931.
- Eadie, J. M., Sherman, P. & Semel, B. 1998: Conspecific brood parasitism, population dynamics, and the conservation of cavity-nesting species. In: Behavior Ecology and Conservation Biology (Caro, T., ed.). Oxford Univ. Press, Oxford, pp. 306–340.
- Falchuk, K. H., Contin, J. M., Dziedzic, T. S., Feng, Z., French, T. C., Heffron, G. J. & Montorzi, M. 2002: A role for biliverdin IX α in dorsal axis development of *Xenopus laevis* embryos. *Proc. Natl Acad. Sci. U. S. A.* **99**, 251–256.
- Gowaty, P. A. & Bridges, W. C. 1991: Nestbox availability affects extra-pair fertilizations and conspecific nest parasitism in eastern bluebirds, *Sialia sialis*. *Anim. Behav.* **41**, 661–675.
- Gowaty, P. A. & Karlin, A. A. 1984: Multiple paternity and paternity in single broods of monogamous eastern bluebirds (*Sialia sialis*). *Behav. Ecol. Sociobiol.* **15**, 91–95.
- Gowaty, P. A. & Plissner, J. 1998: Eastern bluebird, *Sialia sialis*. In: The Birds of North America, No 381 (Poole, A. & Gill, F., eds). The Birds of North America, Inc., Philadelphia, pp. 1–32.
- Gowaty, P. A. & Wagner, S. G. 1988: Breeding season aggression of female and male eastern bluebirds (*Sialia sialis*) to models of potential conspecific and interspecific egg dumpers. *Ethology* **78**, 238–250.
- Gowaty, P. A., Plissner, J. H. & Williams, T. G. 1989: Behavioral correlates of uncertain parentage: mate guarding and nest guarding by eastern bluebirds, *Sialia sialis*. *Anim. Behav.* **38**, 272–284.
- Hunt, S., Kilner, R. M., Langmore, N. E. & Bennett, A. D. T. 2003: Conspicuous, ultraviolet-rich mouth colours in begging chicks. *Proc. R. Soc. Lond. B Biol. Sci.* **270**, S25–S28.
- Jackson, W. M. 1998: Parasitic Birds and Their Hosts: Studies in Coevolution. Oxford Univ. Press, Oxford.
- Kennedy, G. Y. & Veevers, H. G. 1976: A survey of avian eggshell pigments. *Comp. Biochem. Physiol. B* **55**, 117–123.
- Lyon, B. E. 1993: Tactics of parasitic American coots: host choice and the pattern of egg dispersion among host nests. *Behav. Ecol. Sociobiol.* **33**, 87–100.
- Lyon, B. E. 2003: Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature* **422**, 495–499.
- Lyon, B. E., Hochachka, W. M. & Eadie, J. M. 2002: Paternity-parasitism trade-offs: a model and test of host-parasite cooperation in an avian conspecific brood parasite. *Evolution* **56**, 1253–1266.
- Møller, A. P. & Petrie, M. 1991: Evolution of intraspecific variability in bird's egg: Is intraspecific nest parasitism the selective agent? *Proc. Int. Ornithol. Congr.* **20**, 1041–1048.
- McGraw, K. J. 2005: The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? *Anim. Behav.* **96**, 757–764.
- Meek, S. B. & Robertson, R. J. 1995: Time of day of egg laying by eastern bluebirds. *Wilson Bull.* **107**, 377–379.

- Moreno, J., Osorno, J. L., Morales, J., Merino, S. & Tomás, G. 2004: Egg colouration and male parental effort in the pied flycatcher *Ficedula hypoleuca*. *J. Avian Biol.* **35**, 300—304.
- Moreno, J., Morales, J., Lobato, E., Merino, S., Tomás, G. & Martínez-de la Puente, J. 2005: Evidence for the signaling function of egg color in the pied flycatcher *Ficedula hypoleuca*. *Behav. Ecol.* **16**, 931—937.
- Rothstein, S. I. 1982: Mechanisms of avian egg recognition: which egg parameters elicit responses by rejecter species? *Behav. Ecol. Sociobiol.* **11**, 229—239.
- Schluter, D. & Price, T. 1993: Honesty, perception and population divergence in sexually selected traits. *Proc. R. Soc. Lond. B Biol. Sci.* **253**, 117—122.
- Siefferman, L. & Hill, G. E. 2005: Evidence for sexual selection on structural plumage coloration of female eastern bluebirds. *Evolution* **59**, 1819—1828.
- Siefferman, L., Navara, K. & Hill, G. E. 2006: Female condition is correlated with egg coloration in eastern bluebirds. *Behav. Ecol. Sociobiol.* **59**, 651—656.
- SPSS 2004: SPSS 11.5 for Windows. SPSS, Chicago.
- Trivers, R. L. 1972: Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Campbell, B., ed.). Adeline Press, Chicago, pp. 136—179.