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RESEARCH ARTICLE

Intraclutch variation in egg appearance of Brown-headed Cowbird hosts

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ABSTRACT

Several species of avian brood parasites have evolved egg mimicry, which can interfere with host egg rejection. Parasitic egg mimicry may select for decreased intraclutch variation in host egg appearance to facilitate the recognition and rejection of parasitic eggs. This hypothesis has received scant attention in hosts of the Brown-headed Cowbird (*Molothrus ater*) because the cowbird apparently has not evolved mimetic eggs. Nonetheless, hosts with eggs similar in appearance to cowbird eggs should minimize intraclutch variation to increase the likelihood of detecting parasitism. By contrast, there may be minimal selection pressure to reduce intraclutch variation in hosts with eggs that are divergent from cowbird eggs. Using reflectance spectrometry, we compared the intraclutch variation between accepters and rejecters of 2 groups of host species: those with eggs similar in appearance to cowbird eggs (white maculate eggs) and those with eggs that clearly diverge in appearance from cowbird eggs (blue eggs). We predicted that rejecters with white maculate eggs should have lower intraclutch variation than accepters, whereas accepters and rejecters with blue eggs should have similar amounts of intraclutch variation. The intraclutch variation between accepters and rejecters with blue eggs did not differ, which matched our predictions. However, rejecters with white maculate eggs did not consistently have lower intraclutch variation than accepters; thus, our hypothesis was not supported for this group. A more comprehensive study, focused on cowbird hosts nesting in grassland and edge habitats, is warranted to determine whether a pattern between intraclutch variation and egg rejection exists among hosts with white maculate eggs.

Keywords: brood parasitism, Brown-headed Cowbird, egg recognition, egg rejection, intraclutch egg variation, *Molothrus ater*

Variación en la apariencia de los huevos dentro de la nidada de aves parasitadas por *Molothrus ater*

RESUMEN

Muchas especies de aves parásitas de cría han evolucionado el mimetismo de sus huevos, lo que puede interferir en el rechazo de los huevos por parte del hospedero. El mimetismo de los huevos del parásito podría seleccionar hacia una disminución de la variación en la apariencia de los huevos dentro de la nidada del hospedero, para facilitar el reconocimiento y el rechazo de los huevos del parásito. Esta hipótesis ha recibido escasa atención en los hospederos de *Molothrus ater*, debido a que éste aparentemente no ha evolucionado huevos miméticos. Sin embargo, los hospederos con huevos en apariencia similares a los de *M. ater* deberían minimizar la variación dentro de la nidada para aumentar la probabilidad de detectar el parasitismo. En contraste, debería haber mínimas presiones de selección para reducir la variación dentro de la nidada en hospederos cuyos huevos son diferentes a los de *M. ater*. Usando espectrometría de reflectancia, comparamos la variación dentro de la nidada entre aves que rechazan y que no rechazan los huevos del parásito, en dos grupos de especies hospedero: aquéllos con huevos aparentemente similares a los de *M. ater* (huevos blanco inmaculado), y aquéllos que en apariencia son claramente divergentes de los de *M. ater* (huevos azules). Predecimos que los huéspedes que rechazan los huevos del parásito y que tienen huevos blancos deberían mostrar menor variación dentro de la nidada que aquellos que no los rechazan, mientras que las aves con huevos azules que los rechazan y las que no los rechazan deberían tener una cantidad similar de variación dentro de la nidada. La variación dentro de la nidada no fue diferente entre las aves de huevos azules que rechazan y no rechazan los huevos del parásito, lo que concuerda con nuestras predicciones. Sin embargo, las aves de huevos blancos que rechazan los huevos del parásito no tuvieron consistentemente menor variación dentro de la nidada que las aves que no los rechazan, de modo que no tuvimos sustento para nuestra hipótesis dentro de este grupo. Un estudio más exhaustivo que se enfoque en los hospederos de *M. ater* que anidan en pastizales y en hábitats de borde es necesario para determinar si existe un patrón entre la variación de los huevos dentro de la nidada de hospederos con huevos blancos y el rechazo de los huevos del parásito.

Palabras clave: Molothrus ater, parasitismo de cria, rechazo de huevos, reconocimiento de huevos, variación de los huevos dentro de la nidada

INTRODUCTION

Obligate avian brood parasites lay their eggs in the nests of other species and have evolved adaptations that allow them to trick the hosts into raising their young (Davies 2000). The Brown-headed Cowbird (*Molothrus ater*; hereafter "cowbird") is a generalist brood parasite known to have parasitized >240 host species (Lowther 2011). Brood parasitism is costly for the host because, in many cases, cowbird nestlings outcompete host young for food (Lichtenstein and Sealy 1998, Rivers 2007), and even large hosts capable of raising their offspring with a cowbird nestling may incur costs due to egg removal by cowbirds (Sealy 1992, Peer 2006, Croston and Hauber 2014). Therefore, it is expected that hosts should evolve defenses against brood parasitism, such as egg ejection, whereby the host removes the foreign egg from the nest and continues incubating its own eggs (Rothstein 1975, Peer and Sealy 2004). However, only ~10% of cowbird hosts are known to eject cowbird eggs (Rothstein 1975, Peer and Sealy 2004).

Several parameters might affect a host's ability to recognize a foreign egg in the nest. Among these are size, ground color, maculation (spotting) pattern of the egg (Rothstein 1982, Spottiswoode and Stevens 2010), the amount of ultraviolet (UV) light reflecting off the egg (Cherry and Bennett 2001, Avilés et al. 2006), the amount of light present in the nest (Peer et al. 2006, Avilés 2008, Langmore et al. 2009), and the amount of variation in egg appearance or size in eggs within a clutch (intraclutch variation) or between clutches of a population (interclutch variation) (Øien et al. 1995). High intraclutch variation in egg appearance may reduce a host's ability to recognize and reject a parasitic egg (Moskát et al. 2008, Peer et al. 2010), especially in a system where the brood parasite lays mimetic eggs (Stokke et al. 2002, 2007). The Common Cuckoo (*Cuculus canorus*) is known for its convincing egg mimicry, and this hypothesis has been tested several times in its hosts. Some studies have found a negative correlation between rejection frequency and intraclutch variation (Soler and Møller 1996, Stokke et al. 1999, Soler et al. 2000, Stevens et al. 2013), whereas others found no correlation (Karcza et al. 2003, Procházka and Honza 2003, Lovászi and Moskát 2004, Bán et al. 2013) or a positive correlation (Cherry et al. 2007).

Few studies have considered intraclutch variation in egg appearance and its effect on egg rejection in hosts of the Brown-headed Cowbird, because cowbird eggs are non-mimetic for the majority of hosts (Stokke et al. 2002, Underwood and Sealy 2008; but see Peer et al. 2000). Rejecter hosts with eggs that resemble cowbird eggs should evolve minimal intraclutch egg variation to increase the likelihood of detecting parasitism (Stokke et al. 1999). Likewise, there should be decreased selection pressure to evolve low intraclutch variation for both rejecter and accepter species with egg types that are clearly divergent from cowbird eggs (Stokke et al. 2002, 2007). To test these hypotheses, we compared the intraclutch variation in egg appearance between rejecters and accepters with eggs that resemble cowbird eggs (white maculate eggs) and between rejecters and accepters with eggs that are more divergent from cowbird eggs (blue eggs), at least to human observers. We used reflectance spectrometry and avian visual modeling to estimate intraclutch variation through a bird's visual perspective. We predicted that (1) rejecters with eggs similar to those of the cowbird would have lower intraclutch variation than accepters with eggs that resemble cowbird eggs and (2) rejecters and accepters with eggs divergent from cowbird eggs would have similar levels of intraclutch variation.

METHODS

Spectral reflectance of the eggs of 11 cowbird hosts was measured in 2011 using eggs from the Field Museum in Chicago, Illinois, USA. Eggs were collected from throughout the United States. Egg color is known to fade over time, especially in the blue-green chroma, and the storage of eggs in museum collections may have an effect on the UV chroma and overall brightness (Cassey et al. 2010a, 2012). Therefore, all eggs measured in the present study were collected from roughly the same period (1886–1922). In addition, because we compared only the variation within clutches, any fading of pigments should be similar among all eggs.

Five complete clutches from each host were measured, and hosts were chosen on the basis of 2 criteria: their response to Brown-headed Cowbird parasitism (accepter, intermediate rejecter, or rejecter) and egg coloration (white maculate or blue), as perceived by human eyes. Rejecters remove ≥75% of cowbird eggs laid in their nests, intermediate rejecters remove 21–74% of cowbird eggs, and accepters remove ≤20% of cowbird eggs (Peer and Sealy 2004). Brown-headed Cowbird eggs have a whitish ground color with brown, reddish brown, or gray round spots. Studies have demonstrated that birds can use ground color and spotting pattern as cues for rejecting eggs (Rothstein 1982, Honza et al. 2007, Moskát et al. 2010, de la Colina et al. 2012). Therefore, we focused on 3 variables when determining whether eggs should be considered similar in appearance to cowbird eggs (white maculate category) versus clearly divergent from cowbird eggs (blue category): ground color, spotting color, and the type of maculation (immaculate, round spots, or long, scrawling lines). Eggs placed in the white maculate category included hosts with similar ground color to the cowbird egg (white), similar spotting color (brown, red, or gray spots), and similar maculation (spots) (Figure 1). Eggs placed in the blue category had a blue ground color, and 3

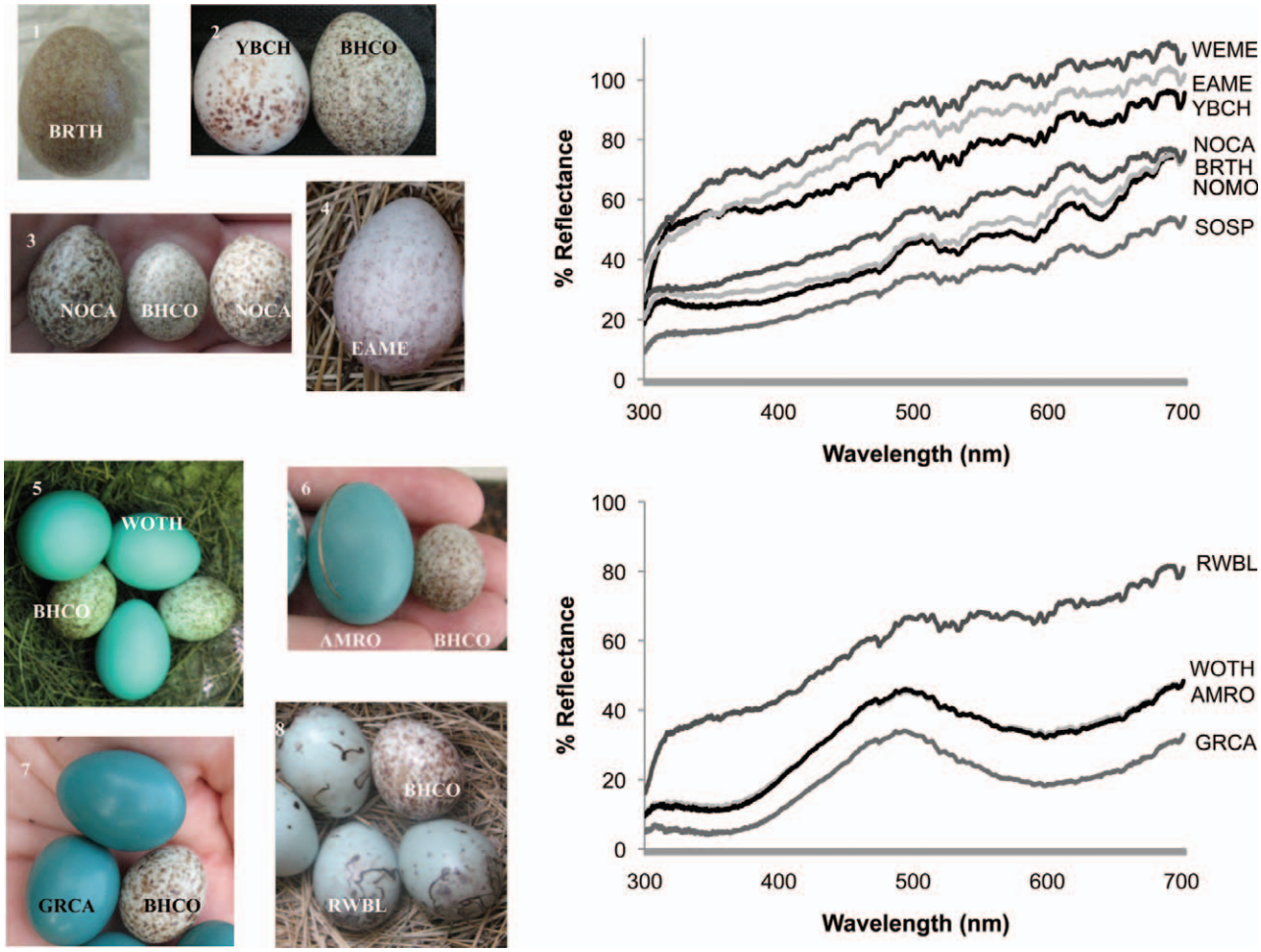

FIGURE 1. Fresh eggs representing the host eggs measured from the Chicago Field Museum's egg collection, with the average spectral reflectance of eggs from each species measured (top: hosts with white maculate eggs; bottom: hosts with blue eggs). Pictures 1–4 are examples of eggs placed in the white maculate egg category (host eggs similar in appearance to Brown-headed Cowbird eggs), and pictures 5–8 are examples of eggs placed in the blue egg category (host eggs clearly divergent in appearance from Brown-headed Cowbird eggs). The average spectra for each species was calculated using the spectra of 1 randomly chosen egg from each clutch measured (a total of 5 eggs for each species). Abbreviations: BRTH = Brown Thrasher, YBCH = Yellow-breasted Chat, NOCA = Northern Cardinal, EAME = Eastern Meadowlark, BHCO = Brown-headed Cowbird, WOTH = Wood Thrush, AMRO = American Robin, GRCA = Gray Catbird, RWBL = Red-winged Blackbird, NOMO = Northern Mockingbird, SOSP = Song Sparrow, and WEME = Western Meadowlark. All pictures were taken by V. E. Abernathy and B. D. Peer.

hosts had immaculate eggs, whereas 1 (Red-winged Blackbird [*Agelaius phoeniceus*]) had eggs with black scrawls (Figure 1). Therefore, eggs placed in the blue category differed from cowbird eggs in all 3 variables.

Rejecter species with white maculate eggs included Brown Thrasher (*Toxostoma rufum*) and Western Meadowlark (*Sturnella neglecta*); the intermediate rejecters were Northern Mockingbird (*Mimus polyglottos*) and Eastern Meadowlark (*S. magna*); and the accepters were Yellow-breasted Chat (*Icteria virens*), Song Sparrow (*Melospiza melodia*), and Northern Cardinal (*Cardinalis cardinalis*). Rejecters with blue eggs included American Robin (*Turdus migratorius*) and Gray Catbird (*Dumetella carolinensis*), and accepters included Wood Thrush (*Hylocichla mustelina*) and Red-winged Blackbird. There are few known intermediate rejecters, the majority of which have white maculate eggs (Peer and Sealy 2004); thus, there was no intermediate-rejecter response category for hosts with blue eggs. The Wood Thrush is an evolutionarily recent cowbird host in relation to the other hosts used in the present study (Peer and Sealy 2004), which may have an effect on its intraclutch variation simply because it has had less exposure time to cowbirds than the other species. To reduce confounding effects due to phylogenetic relatedness among host species (Cassey et al. 2010b), all response and egg-color categories contain species from different families.

Spectral reflectance was measured using a USB4000 Fiber Optic Spectrometer and SpectraSuite 2008 software

with a PX-2 xenon light source and a WS-1-SL white reflectance standard (Ocean Optics, Dunedin, Florida, USA). The light probe had a diameter of 0.4 mm, was held inside a probe holder at a 45° angle, and was placed directly onto the surface of the egg. Eggs were divided into 3 regions (cap, middle, and blunt end), and 3 measurements were taken in random areas within each egg region (see Honza and Polačiková 2008). These 9 measurements were then averaged together to obtain the average spectral reflectance for the entire egg. We did not distinguish between ground color and spotting when taking measurements, because most of the white maculate eggs had spotting that covered the majority of the egg and only ground color can be measured for the immaculate eggs. A light and a dark reference were taken before a new egg region was measured (3 total light and dark references for each egg) to ensure more accurate results in case of any drifting in the spectrometer. All measurements were taken under a black cloth in the dark to reduce noise from ambient light (Underwood and Sealy 2008). Spectral reflectance for each egg was recorded from 300 to 700 nm. In the present study, we focused on variation in egg color, but birds with maculated eggs likely use a combination of the ground color and spotting pattern to recognize their eggs (Spottiswoode and Stevens 2010). However, we did not have standardized photographs or other sources of measurements for a quantitative assessment of maculation (Stoddard and Stevens 2010).

We analyzed our data using the pavo package (Maia et al. 2013) in the R Statistical Package (R Development Core Team 2010). Pavo provides a method of analyzing spectral reflectance measurements and representing color through bird vision on the basis of current information known about avian visual systems and cone-type sensitivities. Birds have 4 color-sensitive retinal cones and 2 visual systems: ultraviolet sensitive cone-types (UVS) or violet sensitive cone-types (VS) (Ödeen et al. 2011). The UVS system appears to be the most prevalent system in the Passerida clade, which includes every family tested in the present study (Ödeen et al. 2011). Further, Aidala et al. (2012) found that 6 of the host species and close relatives of some of the other hosts used in the present study all have the UVS-system. Therefore, we performed our analysis using the UVS-visual system known in Blue Tits (*Cyanistes caeruleus*).

Statistical Analyses

Intraclutch variation in egg appearance was estimated by calculating the just noticeable differences (JND) between individual eggs within each clutch using pavo in R. Just noticeable differences represent how distinguishable one egg is from another on the basis of its spectral reflectance and the type of visual system being used in the analysis (Avilés 2008, Cassey et al. 2008). Values <1 are considered to represent indistinguishable differences, and values >1 represent distinguishable differences. Therefore, the higher the JND value, the more distinguishable the two egg colors should be from one another (Siddiqi et al. 2004; also see Spottiswoode and Stevens 2010).

The mean JND of each clutch was calculated on the basis of the individual JND values within each clutch. Mean JND values were compared using an analysis of variance (ANOVA), first among response categories within each egg category (white maculate eggs: accepter, $n = 15$ clutches; intermediate rejecter, $n = 10$ clutches; rejecter $=$ 10 clutches; blue eggs: accepter, $n = 10$ clutches; rejecter, $n = 10$ clutches) and next among host species within each egg category (white maculate eggs: $n = 7$ hosts, $n = 5$ clutches host^{-1}; blue eggs: $n = 4$ hosts, $n = 5$ clutches host^{-1}). If the ANOVA showed significant differences, Tukey's post hoc tests were performed. In the case of the blue egg category, a Welch's t-test was performed for the comparison between accepters and rejecters. Shapiro-Wilk tests were run on the mean JND values for each species and response category, and the data were determined to be normal. Statistical analyses were performed with the R Statistical Package (R Development Core Team 2010), and all tests were two-tailed with an alpha value of 0.05.

RESULTS

There were no significant differences in intraclutch variation between the 3 response categories for hosts with white maculate eggs (accepter: $n = 15$ clutches, $n = 5$ clutches each of Yellow-breasted Chat, Northern Cardinal, and Song Sparrow; intermediate rejecter: $n = 10$ clutches, $n = 5$ clutches each of Northern Mockingbird and Eastern Meadowlark; rejecter: $n = 10$ clutches, $n = 5$ clutches each of Brown Thrasher and Western Meadowlark) (ANOVA, $F_{2, 32} = 1.52$, $P = 0.23$). When the 7 species with white maculate eggs were compared against each other ($n = 5$ clutches species^{-1}), there were several significant differences (ANOVA, $F_{6, 28} = 12.78$, $P < 0.001$; Figure 2). Northern Mockingbird, Song Sparrow, and Northern Cardinal had significantly higher intraclutch variation than Yellow-breasted Chat and the meadowlarks. Intraclutch variation in Brown Thrasher was also significantly higher than that in Yellow-breasted Chat, but not significantly higher than that in the meadowlarks.

There were no significant differences in intraclutch variation between accepters ($n = 10$ clutches, $n = 5$ clutches each of Wood Thrush and Red-winged Blackbird) and rejecters ($n = 10$ clutches, $n = 5$ clutches each of American Robin and Gray Catbird) with blue eggs (t-test, $t = -1.67$, $P = 0.12$). When the 4 hosts with blue eggs were individually compared (5 clutches for each species), there were no significant differences in intraclutch variation (ANOVA, $F_{3, 16} = 1.97$, $P = 0.16$; Figure 3).

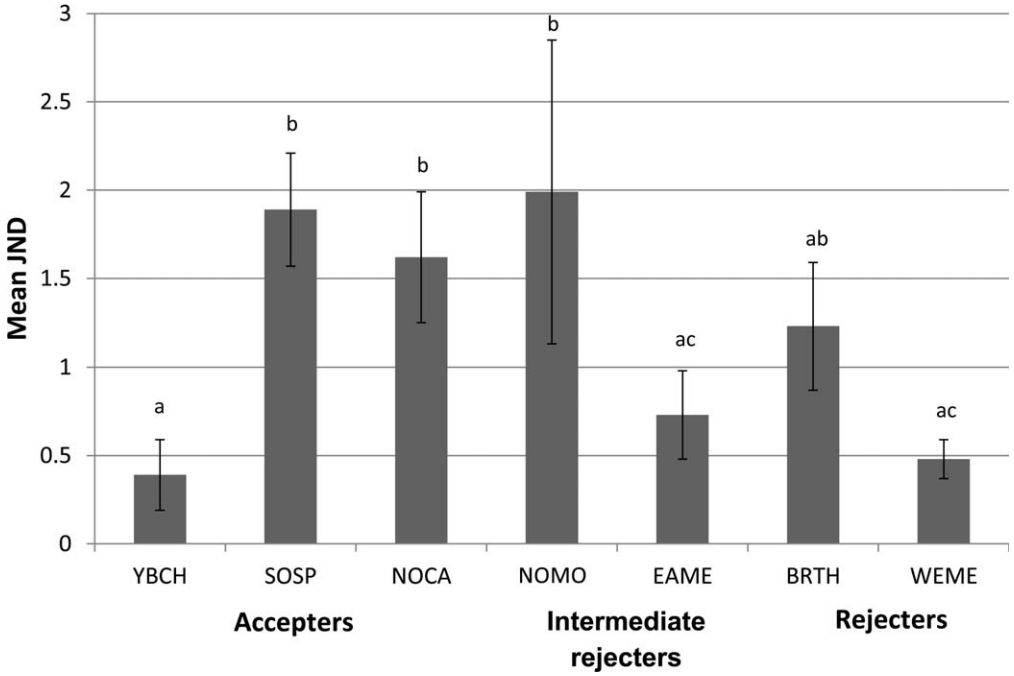

FIGURE 2. Intraclutch variation of host species with white maculate eggs represented by mean just noticeable differences (JND) ± SD of clutches (5 clutches per species; ANOVA, Tukey, $P \leq 0.05$). JND values were calculated using the pavo package (Maia et al. 2013) in the R Statistical Package (R Development Core Team 2010). The dataset was analyzed in the UVS Blue Tit visual system. Significant differences are indicated by different letters. Abbreviations: NOMO = Northern Mockingbird, BRTH = Brown Thrasher, YBCH = Yellow-breasted Chat, SOSP = Song Sparrow, NOCA = Northern Cardinal, EAME = Eastern Meadowlark, and WEME = Western Meadowlark.

We took 9 spectral reflectance measurements at random locations on each egg within 3 egg regions. For maculated eggs, if the spotting or scrawling on the egg is randomly distributed, these measurements should show high repeatability. Therefore, we performed a Pearson's correlation coefficient repeatability analysis comparing all measurements for each egg (Lessells and Boag 1987, Avilés et al. 2004, Polačiková et al. 2007). There was significant repeatability and generally high repeatability for the majority of eggs ($0.77 \leq r \leq 1.0$, $P < 0.001$), although correlation coefficients were lower for 1 Song Sparrow egg and several Yellow-breasted Chat eggs (Pearson's correlation coefficients for all eggs: $0.60 \leq r \leq 1.0$, $P < 0.001$, $n = 225$ eggs; Table 1). Therefore, further repeatability tests between and within egg regions (cap, middle, and blunt end) were performed for all eggs where $r < 0.75$. There was significantly high repeatability among the 3 reflectance measurements within an egg region for each egg (Pearson's correlation coefficients: $0.82 \leq r \leq 1.0$, $P < 0.001$, $n = 9$ eggs); but for 2 Yellow-breasted Chat eggs, $0.7 \leq r \leq 0.72$ between the cap and blunt end and between the middle and blunt end ($P < 0.001$). This result is not surprising, because some maculated eggs have heavier spotting on the blunt end. Harper (1994) considered repeatabilities between 0.70 and 0.90 to be high. Therefore, though

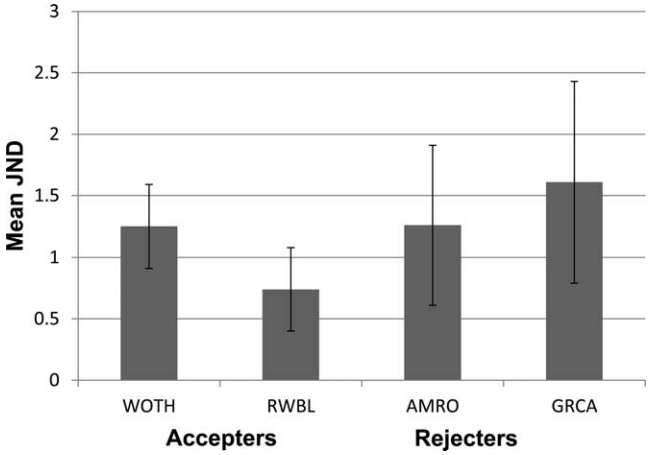

FIGURE 3. Intraclutch variation of host species with blue eggs represented by mean just noticeable differences (JND) ± SD of clutches (5 clutches per species; ANOVA, Tukey, $P \leq 0.05$). JND values were calculated using the pavo package (Maia et al. 2013) in the R Statistical Package (R Development Core Team 2010). The dataset was analyzed in the UVS Blue Tit visual system. There were no significant differences among host species. Abbreviations: WOTH = Wood Thrush, AMRO = American Robin, GRCA = Gray Catbird, and RWBL = Red-winged Blackbird.

TABLE 1. The estimated repeatability of the 9 spectrophotometric reflectance measurements taken for each egg (Pearson's correlation coefficient) with a summary of the *r* and *P* values calculated for all the eggs of each species (*n* = number of eggs measured for each species).

Species	*r*	*P*	*n*
Wood Thrush[a]	≥0.97	<0.001	20
American Robin[b]	≥0.99	<0.001	17
Gray Catbird[c]	≥0.96	<0.001	20
Northern Mockingbird[d]	≥0.77	<0.001	18
Brown Thrasher[e]	≥0.87	<0.001	22
Yellow-breasted Chat[f]	≥0.60	<0.001	23
Song Sparrow[g]	≥0.74	<0.001	22
Northern Cardinal[h]	≥0.79	<0.001	16
Red-winged Blackbird[i]	≥0.92	<0.001	19
Eastern Meadowlark[j]	≥0.79	<0.001	23
Western Meadowlark[k]	≥0.81	<0.001	25

[a] Eggs collected from Kansas and West Virginia in 1888, 1898, 1906, and 1908.
[b] Eggs collected from Illinois and West Virginia in 1893, 1901, 1904, and 1914.
[c] Eggs collected from Illinois, New York, and West Virginia in 1897, 1904, and 1906.
[d] Eggs collected from Arizona, Arkansas, and California in 1898, 1900, and 1903.
[e] Eggs collected from Illinois in 1886, 1889, 1893, and 1895.
[f] Eggs collected from Illinois, Iowa, and West Virginia in 1898, 1902, 1904, 1905, and 1906.
[g] Eggs collected from Connecticut, New York, and West Virginia in 1887, 1894, 1899, and 1907.
[h] Eggs collected from Alabama, Illinois, North Carolina, and Washington, DC, in 1888, 1895, 1898, and 1900.
[i] Eggs collected from Arizona and Illinois in 1893, 1896, 1898, and 1922.
[j] Eggs collected from Connecticut and Illinois in 1891, 1893, and 1910.
[k] Eggs collected from California, Colorado, Kansas, and Minnesota in 1890, 1893, 1894, 1900, and 1910.

repeatabilities showed some variation, our measurements for a single egg are still comparable.

DISCUSSION

Hosts with White Maculate Eggs

Significant differences in intraclutch variation occurred at the species level for hosts with white maculate eggs, and half fit the hypothesized predictions. The Song Sparrow and Northern Cardinal (both accepters) had higher intraclutch variation than the Eastern Meadowlark (an intermediate rejecter) and the Western Meadowlark (a rejecter) (Figure 2). Although Northern Cardinals typically accept cowbird eggs, which are very similar in appearance to their own (V. E. Abernathy and B. D. Peer personal observation), they are capable of rejecting white eggs (Burhans and Freeman 1997). However, the cost of parasitism for cardinals is relatively low because their incubation period is similar to that of cowbirds and are a larger host (Eckerle and Breitwisch 1997). Therefore, selection for reduced intraclutch egg variation may be minimal, which may explain, in part, why they accept cowbird eggs.

The Yellow-breasted Chat did not fit the prediction of an accepter having high intraclutch egg variation, which suggests that low intraclutch variation may not be enough to facilitate rejection in chats. Chats are considered accepters of cowbird eggs (Rohwer and Spaw 1988), but they eject unspotted eggs (Burhans and Freeman 1997). Chat eggs can be difficult to distinguish from cowbird eggs, and chats occasionally make recognition errors when attempting to eject cowbird eggs (Burhans and Freeman 1997), which could also have selected for low intraclutch variation in this commonly parasitized host (Friedmann 1963).

The Northern Mockingbird (an intermediate rejecter) had the highest amount of intraclutch variation, and the Brown Thrasher (a rejecter) had an intermediate amount, both of which did not fit our predictions. This could indicate that intraclutch variation may not affect rejection in these hosts or that increased intraclutch variation aids in rejection (Cherry et al. 2007). If the host is able to learn the appearance of each of its highly variable eggs, this could allow it to better recognize a cowbird egg (Tibbetts and Dale 2007). Brown Thrashers are one of the few North American hosts that exhibit geographic variation in their response to parasitism (Elliott 1978, Haas and Haas 1998); thus, the lower level of rejection in some locations could be a consequence of the constraint of intraclutch egg variation we document here. Likewise, it could be a result of misimprinting on cowbird eggs in areas where parasitism frequencies are high (Haas and Haas 1998; also see Strausberger and Rothstein 2009).

Hosts with Blue Eggs

Hosts with blue eggs did not show significant differences in either comparison, and there was no apparent relationship between intraclutch variation and egg rejection. This supports our second prediction that accepters and rejecters with eggs that are clearly different from cowbird eggs should have similar amounts of intraclutch variation. Selection pressure for low intraclutch variation in these hosts may be minimal because these eggs are readily distinguishable from cowbird eggs (Stokke et al. 2002, 2007). However, Peer et al. (2010) found that high intraclutch variation constrained egg rejection in the Common Grackle (*Quiscalus quiscula*), despite the fact that this species lays eggs that are obviously different from cowbird eggs.

Acceptance of cowbird parasitism by the Wood Thrush is probably an example of evolutionary lag (Rothstein 1975).

The Wood Thrush, which tends to inhabit dense forests, could be a relatively new host, because forests have become more fragmented and may not have yet developed the ability to reject foreign eggs (Peer and Sealy 2004). The lack of egg rejection by the Red-winged Blackbird is enigmatic. This species has likely been parasitized for a long period because it occupies the same habitat as the cowbird, has nonmimetic eggs (Peer and Sealy 2004), and, according to this study, tends to have low intraclutch variation. Nevertheless, the Red-winged Blackbird is a larger host capable of raising its young successfully with a cowbird nestling, so selection pressures to evolve egg recognition may also be lower in this host (Clotfelter and Yasukawa 1999; also see Grayson et al. 2013). Additionally, in some Red-winged Blackbird populations, individuals can be repeatedly parasitized, which can also reduce the benefits of egg ejection (Hoover et al. 2006; but see Ward et al. 1996).

Conclusions

Each host has a unique history of cowbird parasitism, and selection pressures for circumventing parasitism vary among and within host populations (Rothstein 1990). In addition, environmental factors and selection pressures other than brood parasitism can affect eggshell color and maculation pattern of a species' eggs, and, therefore, the amount of intraclutch variation in a host may not be the direct result of brood parasitism (Cherry and Gosler 2010). The results of our study suggest that the degree of intraclutch variation in a particular Brown-headed Cowbird host may not be sufficient to predict rejection frequency. Rather, several factors, such as length of exposure to cowbird parasitism, the costs associated with parasitism, and geographic location, along with intraclutch variation, may affect a species' response to cowbird eggs. A more comprehensive study of species with white maculate eggs is warranted, especially focusing on grassland and edge species that have been exposed to cowbird parasitism for a historically long period and that have eggs that appear similar to cowbird eggs (Peer et al. 2000).

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