Differences in morphology and colour pattern of shiny cowbird (Molothrus bonariensis) eggs found in nests of two hosts

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Genetic differentiation among shiny cowbird (Molothrus bonariensis) females that use different hosts indicates that in this brood parasite, host use is not random at an individual level. We tested whether there exist differences in morphology and coloration between eggs of shiny cowbirds laid in the nests of two different hosts, the chalk-browed mockingbird (Mimus saturninus) and the house wren (Troglodytes aedon). We took morphometric measures of shiny cowbird eggs found in nests of mockingbirds and wrens and analysed their coloration using digital photography and reflectance spectrometry. We found that shiny cowbird eggs found in mockingbird nests were wider and more asymmetric than those found in wren nests. In addition, cowbird eggs coming from mockingbird nests were brighter and had higher relative red reflectance than those coming from wren nests. Our results show that shiny cowbird eggs laid in nests of two different hosts vary in shape and background colour, but not in spotting pattern. © 2011 The Linnean Society of London, Biological Journal of the Linnean Society, 2011, 102, 838–845.

ADDITIONAL KEYWORDS: brood parasitism – egg morphology – eggshell spotting.

INTRODUCTION

Avian obligate brood parasites are completely dependent on other species, the hosts, to raise their offspring. The co-evolutionary arms race between parasites and hosts may favour the evolution of host parasitic defences such as egg or chick discrimination that may in turn be counterbalanced by the parasites (Davies & Brooke, 1989; Davies, Bourke & Brooke, 1989; Rothstein, 1990; Rothstein & Robinson, 1998; Davies, 2000). In the common cuckoo Cuculus canorus, a host-generalist brood parasite, this co-evolutionary process has led to female lineages becoming host specialists and evolving mimetic eggs that resemble those of particular hosts (Brooke & Davies, 1988; Moksnes & Røskaft, 1995; Gibbs et al., 2000; Avilés, 2008; Stoddard & Stevens, 2010). In this way, individual common cuckoos minimize egg losses as a result of rejection in its many different host species.

Most brood parasitic cowbirds (Molothrus spp.) have eggs of polymorphic coloration (Ortega, 1998). Among them, the shiny cowbird (Molothrus bonariensis), a highly generalist brood parasite that uses more than 240 species as hosts (Friedmann & Kiff, 1985; Ortega, 1998; Lowther & Post, 1999), shows eggs with extreme variation in their colour pattern, not only in background colour, but also in spotting density (Fig. 1). Background colours can be pure white, light bluish, greenish white, light cream, dark cream or light brown, while spotting varies from absent to very intense (Hudson, 1874; Friedmann, 1929; Ortega, 1998).

Although polymorphism is very high in shiny cowbirds, egg coloration is considered to be constant for each female (Lyon, 1997). A constant intra-individual eggshell coloration pattern has been found for several bird species (Dufty, 1983; Fleischer, 1985; Collias,
1993; Moksnes et al., 2008) and it has been shown that it is genetically determined (Punnett & Bailey, 1920; Punnett, 1933; Joseph et al., 1999; Gosler, Barnett & Reynolds, 2000; Morales et al., 2010), although environmental factors also seem to play a role in pigment deposition (Avilés et al., 2007). Previous studies have found diverse evidence of genetic control of egg coloration in birds. Collias (1993) found that the inheritance of the background colour in eggs of village weavers (Ploceus cucullatus) is consistent with a model of two autosomal loci, and Hutt (1949) also found autosomal inheritance of egg colour in the domestic chicken (Gallus domesticus). In contrast, Gosler and collaborators (2000) found that the spotting pattern of eggs laid by the Great Tit (Parus major) is consistent with maternal inheritance, although this mechanism of spotting inheritance was not supported by a study in shiny cowbirds (Mahler et al., 2008).

Individual egg colour constancy and heritability of egg coloration, as well as polymorphism at species level, set the grounds for natural selection to act on this trait. Although the shiny cowbird is a host generalist at species level, several studies indicate that individual females do not select nests to lay their eggs randomly, but preferentially parasitize those of only some of the available hosts (Post & Wiley, 1977; Cruz, Manolis & Andrews, 1995; López-Ortiz et al., 2006; De Mársico et al., 2010). In addition, there is genetic differentiation in a mitochondrial molecular marker between shiny cowbird females that parasitize the chalk-browed mockingbird, Mimus saturninus (hereafter mockingbird) and the house wren, Troglodytes aedon (hereafter wren) (Mahler et al., 2007), suggesting non-random host use by females. If egg colour has a genetic basis and there are female lines that parasitize different host species, then genetic drift or a founder effect can lead to colour differentiation between parasite eggs laid in nests of different hosts. In addition, if selection pressures on parasite egg phenotypes among hosts vary, for example because of differences in egg-rejection behaviour (i.e. chalk-browed mockingbirds were reported to reject white immaculate eggs while house wrens accept parasitic eggs of different morphs; Fraga, 1985; Mason, 1986a; Sackmann & Reboreda, 2003; Tuero, Fiorini & Reboreda, 2007), this can lead to egg colour differentiation. Host–parasite co-evolution can also result in egg differentiation in morphology (Antonov et al., 2010) and eggshell strength (Spottiswood, 2010) among host-specific parasite lineages.

The aim of this study was to determine whether there exist differences in egg morphology and coloration between eggs of shiny cowbirds laid in nests of mockingbirds and wrens in a parasite's population where females that use both hosts differ genetically. If divergent evolution occurred in egg colour and/or morphology between both lineages, and these traits were maternally inherited, we expect to find differences between eggs found in nests of both hosts (i.e. laid by females of different host lineages). We do not expect to find differences in the absence of evolutionary forces acting on these traits or in cases of autosomal inheritance or environmental determination of them.

MATERIAL AND METHODS

EGG SAMPLES

The study site was located in Reserve ‘El Destino’, near the town of Magdalena (35°8’S, 57°23’W), Buenos Aires Province, Argentina. During the breeding season (October–January) 2006–2007 we collected data from shiny cowbird eggs found in nests of mockingbirds and wrens. Both species are highly parasitized in this area, showing parasitism frequencies of 66% (mockingbirds, Fiorini & Reboreda, 2006) and 60% (wrens, Tuero et al., 2007) and numerous multiple parasitism events. Mockingbirds build open nests on shrubs or trees with dense foliage at a height of 1.5–2.5 m. The nest is a large open cup of twigs (outer diameter 20–25 cm) lined with fibres and horsehair. Their eggs are 28.6 ± 0.3 mm in length and 20.4 ± 0.2 mm in width (Fiorini & Reboreda, 2006). Wrens use nest boxes placed in the study area within mockingbird territories, at a height of 1.5–1.8 m, with dimensions of 25 × 17 × 13 cm (height × width × depth) and an entrance hole of 4.5 cm in diameter. They build a cup of twigs lined with feathers and horsehair inside the box. Their eggs are 17.5 ± 0.08 mm in length and 13.1 ± 0.4 mm in width.
(Tuero et al., 2007). Both hosts are insectivorous and overlap their breeding seasons from early October to mid-January. Mockingbirds show very aggressive behaviours against cowbird females that approach their nests (Sackmann & Reboreda, 2003), while wrens do not show any agonistic behaviour (Fiorini, 2007).

DATA COLLECTION

In total, we collected 86 and 20 shiny cowbird spotted eggs from mockingbird and wren nests, respectively. We did not include shiny cowbird white immaculate eggs because our aim was to evaluate if there exist differences in background coloration and spotting between parasitic eggs laid in nests of both hosts. White immaculate eggs do not differ and are laid at low frequencies in both hosts (M. A. de la Colina, unpubl. data). Each egg was photographed at the nest using a Nikon Coolpix 4500 digital camera. To standardize and optimize the lighting conditions and position of the eggs, we built a transportable black acrylic box of 10 × 10 × 20 cm (width × depth × height) with a ring of seven light-emitting diodes (LEDs) located on the inside of the top cap. We opted for using LEDs because of their ability to deliver virtually monochromatic light (450 nm), with a very narrow spectrum of reflectance, thus minimizing variations in the source of illumination. We took three photographs per egg: pointed end, blunt end and lateral axis. All photographs in this study were taken with the ‘fine’ quality setting, which has a minimal compression and very small quality loss [it creates an 870 Kb (2272 × 1704 pixels) JPEG file per photo]. We consider that it is unlikely that the storage format used prevented detection of differences between eggs (Stevens et al., 2007).

We studied the following aspects of the eggs: (1) morphometry; (2) coloration; and (3) spotting pattern. We used the software IMAGEJ (Rasband, 1997–2006) to measure length (L) and maximum width (w) of the egg on each photograph. Measures were scaled relative to the ones taken from the egg with a calliper to the nearest 0.1 mm. Calibration error between pixels and centimetres was less than 0.1 mm. These values were used to calculate the egg’s degree of asymmetry

\[ D = \left( R_b - R_p \right) \times \left( L/w^2 \right) \] (Preston, 1968), where \( R_b \) is the radius of curvature at the blunt end and \( R_p \) at the pointed end. We measured colour as the maximum pixel frequency corresponding to each of the primary colour channels (RGB). This measurement was conducted separately on the background and on the spots. To study spotting, we measured three variables within a 6 × 6 mm square: number of spots, total area covered by spots and average spot size. Colour and spotting measures were taken for the three egg regions (pointed end, blunt end and lateral axis).

We also measured colour using reflectance spectrometry. We performed this analysis on shiny cowbird eggshells that had been stored in obscurity at −20 °C for not more than 3 years in the laboratory (11 found in mockingbird nests and 10 in wren nests). We assumed that there was no significant effect of time of collection on reflectance spectra given the close period of collection and the storage method (Soler et al., 2005; Cassey et al., 2010). We measured eggshell reflectance using an Ocean Optics 2000 Spectrometer (Ocean Optics, Inc., Dunedin, FL, USA) with a PX-2 pulsed xenon light source (220–750 nm). Measurements were taken at a 90° angle from a 6-mm diameter area. Reflectance was recorded each 0.35 nm within the avian visible spectrum from 340 to 700 nm using OOIBASE32 software and expressed relative to a white reflection standard of barium sulphate, following Osorio & Ham (2002). We performed three measurements on each egg and took median reflectance values for 3-nm bins. Reflectance values below 340 nm were excluded because of considerable noise at these wavelengths. For each egg, we calculated the average reflectance.

To analyse if parasite eggs tend to mimic host eggs, we also measured coloration on eight mockingbird and 15 wren eggs using the same photographic and spectroscopic procedure. Host eggs were collected from nests where we collected parasite eggs. We took one egg of each host pair’s clutch, thus ensuring that analysed eggs belonged to different females.

STATISTICAL ANALYSIS

We used Mann–Whitney tests to compare morphometric and colour variables between cowbird eggs found in nests of both hosts, as well as between cowbird and host eggs. We performed a principal component analysis (PCA) on background and spot colour summarizing RGB values. We also performed a PCA on the average reflectance values of the eggs (19 reflectance values, taken every 20 nm), obtaining two principal components. The first principal component (PC1) describes variation in brightness (Endler, 1990; Bennett et al., 1997), while the second principal component (PC2) describes variation in spectral shape (Endler, 1990; Endler & Théry, 1996; Cuthill et al., 1999). Reflectance spectra are affected by both spot and background colour, as well as by the percentage of the surface covered by spots. Thus, PC values should not be considered only as background colour, but rather as variables indirectly representing general colour (Martínez-de la Puente et al., 2007). We used one-way ANOVA to compare reflectance of cowbird eggs from different host nests and cowbird and host eggs. We used STATISTICA ver. 6.0 software (StatSoft, 2001) to perform all statistical analyses.
RESULTS

Shiny cowbird eggs found in nests of mockingbirds were wider (mean ± SE: 1.90 ± 0.01 cm) than those found in nests of wrens (1.8 ± 0.01 cm, $Z = -3.05$, $P = 0.002$) and also showed increased asymmetry (mockingbirds: 0.061 ± 0.004; wrens = 0.045 ± 0.006, $Z = -2.66$, $P = 0.007$). Egg length did not differ between hosts (mockingbirds: 2.43 ± 0.01 cm, wrens 2.40 ± 0.02 cm, $Z = -1.12$, $P = 0.26$).

Variation in RGB channels was summarized in one component (PC1) that explained 80% of the variation for background colour (eigenvalue = 2.41) and 94% for spot colour (eigenvalue = 2.82), respectively. In both cases, the loadings of the three variables (red, green and blue) were negative and greater than 0.85. Cowbird eggs from both hosts differed significantly in background colour (Fig. 2A; $Z = 4.42$, $P < 0.001$), but not in spot colour (Fig. 2B; $Z = 1.34$, $P = 0.18$). There were no significant differences in spotting pattern between cowbird eggs from nests of both hosts in any of the studied variables and for any of the three egg regions ($P > 0.2$ for all comparisons).

We found significant differences in reflectance spectra of cowbird eggs found in nests of mockingbirds and wrens, both in brightness and spectral shape (Fig. 3). PC1 (brightness) explained 88% of the variation (eigenvalue = 16.68) and was negatively associated with all wavelengths, whereas PC2 explained 8% of the variation (eigenvalue = 1.60) and was negatively associated with wavelengths between 650 and 700 nm, thus explaining the red colour component. Cowbird eggs found in mockingbird nests were significantly brighter (Fig. 4A; $F_{1,19} = 15.7$, $P < 0.001$) and more reddish (Fig. 4B; $F_{1,19} = 4.18$, $P = 0.05$) than those found in wren nests.

Differences between cowbird eggs found in both hosts were not associated with mimetism to each particular host’s eggs. We found significant differences for most colour variables between cowbird eggs and those of the host in which nest they were found (Fig. 3; Table 1).

DISCUSSION

Our results show that shiny cowbird eggs found in mockingbird nests were wider and more asymmetric than those found in wren nests. Besides, cowbird eggs coming from mockingbird nests were brighter and had higher relative red reflectance than those coming from wren nests.

Egg size and shape differences have also been found among common cuckoos’ host-specific lineages (Antonov et al., 2010). This differentiation might have been driven by host discrimination of poorly

Figure 2. PC1 values summarizing red, blue and green channels for (A) background colour and (B) spot colour of shiny cowbird eggs found in nests of chalk-browed mockingbirds (CBM) and house wrens (HW). The central squares, the large boxes and the bars represent the mean, standard error and standard deviation, respectively. Asterisks represent significant differences (***$P < 0.001$).

Figure 3. Average reflectance spectra of shiny cowbird eggs found in nests of chalk-browed mockingbirds (fine dotted line) and house wrens (continuous line); and hosts’ eggs: chalk-browed mockingbird (long dashes) and house wren (short dashes).
size-mimetic eggs. For the shiny cowbird, it has been suggested that the rejection behaviour of the rufus ovenbird (*Furnarius rufus*) selected for an increase in egg size in parts of its distribution (Mason & Rothstein, 1986). However, none of the two studied hosts rejects parasitic eggs by size (Sackmann & Reboreda, 2003; Tuero et al., 2007), suggesting that egg size differences between shiny cowbird females parasitizing mockingbirds and wrens are unlikely to arise from differences in selection pressures between hosts.

Differences in egg size might appear as a consequence of the variation in the extent of competition for food with nest mates that shiny cowbird chicks face in both hosts (Fiorini, Tuero & Reboreda, 2009). As egg size is positively associated with body mass at hatching (Blomqvist, Johansson & Götmark, 1997) and this, in turn, with the ability to compete for food with nest mates, selective pressures for increasing egg size would be expected in larger hosts where competition for food is more intense, as was found for mockingbirds (Fiorini *et al.*, 2009, D. Tuero, pers. comm.).

Differences in egg size might also have arisen as a result of host nest characteristics. As the holes of natural cavities where wrens nest are mostly very small, this could have impeded the entrance of large shiny cowbirds when this host lineage arose. Considering the allometric relationship of egg size with body size (Brooke & Birkhead, 1991; but see Christians, 2002), smaller eggs should be found in house wren nests. Differences in egg size might also have arisen as a result of host nest characteristics. As the holes of natural cavities where wrens nest are mostly very small, this could have impeded the entrance of large shiny cowbirds when this host lineage arose. Considering the allometric relationship of egg size with body size (Brooke & Birkhead, 1991; but see Christians, 2002), smaller eggs should be found in house wren nests. Asymmetry of eggs is correlated with clutch size-dependent incubation efficiency (Andersson, 1978; Barta & Székely, 1997). Although both hosts vary in clutch size, the final number of eggs in the nest is highly variable because of egg puncture and multiple parasitism by shiny cowbirds. Therefore, it seems unlikely that incubation efficiency is originating the difference in asymmetry between shiny cowbird eggs found in the nests of these hosts.

Differences in eggshell background coloration, however, might be related to hosts’ rejection behaviour. Whereas mockingbirds were reported to reject white immaculate non-mimetic parasitic eggs (Fraga, 1985; Sackmann & Reboreda, 2003), house wrens accept all egg morphs (Mason, 1986a; Kattan, 1997; Tuero *et al.*, 2007). The differently coloured eggs found in nests of both hosts could arise from the rejection of particular morphs by mockingbirds, leaving only eggs of some of the colour patterns in the nests. Alternatively, mockingbird’s rejection behaviour might be a selective factor driving egg colour to a mockingbird-mimetic egg morph in shiny cowbirds that lay in those nests. But there is evidence showing that mockingbirds only reject shiny cowbirds’ white immaculate eggs, accepting all different spotted morphs. Moreover, they accept plaster eggs and other dissimilar eggs, such as those of the screaming

Figure 4. PC1 and PC2 values summarizing reflectance spectra of shiny cowbird eggs found in nests of chalk-browed mockingbirds (CBM) and house wrens (HW). The central squares, the large boxes and the bars represent the mean, standard error and standard deviation, respectively. Asterisks represent significant differences (*$P = 0.05$; ***$P < 0.001$).

Table 1. Colour differences between cowbird eggs found in the nests of a host species and the host’s eggs

<table>
<thead>
<tr>
<th>RGB background colour</th>
<th>Chalk-browed mockingbird</th>
<th>House wren</th>
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<tbody>
<tr>
<td>$Z = 4.55$</td>
<td>$Z = 2.83$</td>
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<td>$P &lt; 0.001$</td>
<td>$P = 0.02$</td>
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<table>
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<tr>
<th>RGB spot colour</th>
<th>Chalk-browed mockingbird</th>
<th>House wren</th>
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<tbody>
<tr>
<td>$Z = -2.11$</td>
<td>$Z = -3.77$</td>
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<tr>
<td>$P = 0.03$</td>
<td>$P &lt; 0.001$</td>
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<tr>
<th>Reflectance spectra</th>
<th>Chalk-browed mockingbird</th>
<th>House wren</th>
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<tbody>
<tr>
<td>$F_{1,17} = 4.16$</td>
<td>$F_{1,23} = 1.28$</td>
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<tr>
<td>$P = 0.05$</td>
<td>$P = 0.27$</td>
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<tr>
<td>$F_{1,17} = 2.11$</td>
<td>$F_{1,23} = 12.6$</td>
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<tr>
<td>$P = 0.16$</td>
<td>$P &lt; 0.01$</td>
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Mann–Whitney Z-values and P-values are shown in the first two rows and one-way ANOVA F-values and P-values in the last two.

PC, principal component; RGB, red, green and blue.

cockbird (*M. rufoaxillaris*; Mason, 1986a; De Mársico & Reboreda, 2008). Besides, in this study, we also failed to find mimicry between shiny cowbird and mockingbird eggs, which suggests that differences in egg colour are more likely explained by divergent evolution of egg colour in both female lineages, not driven by selective pressures of the host but by a founder effect and/or genetic drift. A recent study has shown that population bottlenecks can lead to significant variation in egg morphology causing a differentiation with the source population (Congdon & Briskie, 2010).

We discovered no differences in spotting pattern between shiny cowbird eggs found in both hosts. If spotting was maternally inherited, as suggested by Gosler *et al.* (2000), and divergent evolution on this trait is occurring between females that use different hosts, we expected to find differences in spotting. The absence of differentiation might be a consequence of little selection pressures on eggshell spotting or of non-maternal inheritance, which was also suggested by Mahler *et al.* (2008), who failed to find an association between egg spotting and a molecular marker of maternal inheritance. Also, previous studies found that the eggshell spotting pattern varies according to female condition and/or eggshell thickness (Gosler, Higham & Reynolds, 2005; Sanz & García Navas, 2009). Thus, arrangement of spots on the eggshell might be a plastic trait that is influenced by a female’s nutritional condition and calcium availability.

Heritability of egg characteristics within a lineage implies either maternal inheritance of these traits along each host’s line or, if eggs characteristics are not maternally inherited, assortative mating between individuals raised by the same host, as is the case of the African Vidua finches (Payne, Payne & Woods, 1998; Sorenson, Seč & Payne, 2003). As shiny cowbirds forage in flocks and roost in groups (Ortega, 1998) and no behavioural differences (e.g. vocalizations, habitat use) have been found between individuals, a scenario of assortative mating seems very unlikely in this species. Although inheritance mechanisms of background coloration and egg size need to be further explored in the shiny cowbird, our results are consistent with the hypothesis of host specialization and a maternal inheritance of egg size and background coloration.

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**REFERENCES**


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