

PREFORMATIVE MOLT IN BROOD PARASITIC SCREAMING (*MOLOTHRUS RUFOAXILLARIS*) AND SHINY (*M. BONAERIENSIS*) COWBIRDS

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Resumen. – La muda preformativa en los parásitos de cría Tordo Pico Corto (*Molothrus rufoaxillaris*) y Tordo Renegrido (*M. bonaeriensis*). La muda de las aves neotropicales en general y la de los parásitos de cría de Sudamérica en particular es poco conocida. El Tordo Pico Corto es uno de los parásitos de cría más especializado en el uso de hospedadores utilizando casi exclusivamente al Músico (*Agelaioides badius*) mientras que el Tordo Renegrido es un parásito de cría generalista extremo que utiliza más de 250 hospedadores. Los juveniles del Tordo Pico Corto tienen un plumaje de coloración mimética al del Músico, mientras que los de Tordo Renegrido tienen un plumaje similar a las hembras de su especie. Medimos y analizamos la muda del Tordo Pico Corto y del Tordo Renegrido desde que los pichones salieron del nido (12-14 días de edad) hasta que los juveniles completaron la muda preformativa. No encontramos diferencias sexuales en el patrón de muda de ambas especies. En el Tordo Pico Corto la muda preformativa comenzó antes que en el Tordo Renegrido (37 vs. 61 días de edad) y se inició por las cobertoras internas, siguió por el pecho y resto del cuerpo y finalizó por la cola y cobertoras externas. A su vez, la muda del Tordo Renegrido comenzó por la corona, pecho y dorso, siguió por la garganta y cobertoras externas y finalizó por la cola y cobertoras internas. En ambas especies, la muda de las plumas de vuelo comenzó luego del inicio de la muda de las plumas del cuerpo y comenzó con la muda de las primarias. La edad a la que los juveniles adquirieron el plumaje formativo fue similar en ambas especies (167-181 días). Nuestros resultados muestran que el comienzo de la muda preformativa en el Tordo Pico Corto coincide con la edad en la que los juveniles se independizan de los padres adoptivos y se unen a bandadas de conespecíficos. Esta corta latencia en el inicio de la muda podría favorecer la aceptación de los juveniles del tordo pico corto en bandadas de conespecíficos.

Abstract. – Molt and plumage cycles are poorly known in Neotropical birds in general and in South American brood parasitic cowbirds in particular. The Screaming Cowbird (*Molothrus rufoaxillaris*) is one of the more specialized brood parasites that use almost exclusively the Baywing (*Agelaioides badius*), while the Shiny Cowbird (*M. bonaeriensis*) is an extreme generalist brood parasite that uses more than 250 known hosts. Fledglings of Screaming Cowbird are mimetic in plumage coloration to fledglings of Baywings, while Shiny Cowbird fledglings have female-like plumage. We measured and analyzed plumage changes of Screaming and Shiny Cowbirds since the time of fledging (i.e. 12-14 days of age) until young completed the preformative molt. There were not sexual differences in molting pattern for both species. Screaming Cowbirds started to molt before Shiny Cowbirds (37 vs. 61 days of age). They first molted under-wing coverts, followed by chest, the rest of the body, and finally tail and external coverts. Shiny cowbird molt started at the crown, breast and back, followed by throat, under-wing coverts, and finally upper-wing covers and tail. In both species flight feathers began molting after body feathers and the pattern of molt was similar, starting with the primaries. For both species the preformative molt was completed at the same age (167-181 days). The beginning of the preformative molt in Screaming Cowbirds coincides with the age at which young become independent of their foster parents and join conspecific flocks. This shorter latency to start molting in Screaming Cowbirds could be related to the acceptance of juveniles in conspecific flocks.

Key words: Preformative molt, cowbird, *Molothrus bonaeriensis*, *M. rufoaxillaris*, brood parasitism

INTRODUCTION

The molt is the process of normal and regular growth of feathers through which plumage is partially or completely replaced (Poulin *et al.* 1992, Piratelli *et al.* 2000). Molt is one of the most important processes during the life cycle of birds and although it implies a significant energy cost, it is vital to maintain plumage function through life (Jenni & Winkler 1994). Molt strategies are linked with other aspects of bird's life history, such as breeding cycles, food supply, and in some cases, migration cycles (Jenni & Winkler 1994). Despite this importance, molt remains a relatively little-studied subject in ornithology, in particular in Neotropical species (Thompson & Leu 1995, Rohwer 1999). The majority of the studies on the molt cycle in Neotropical birds (i.e. Piratelli *et al.* 2000, Marini & Durães 2001, Ryder & Wolfe 2009) have focused on the timing of molt in relation to other activities in the annual cycle rather than in the length and extent of the molt and progression of plumages (Ryder & Wolfe 2009).

Birds have relatively similar patterns of molt and descriptive accounts of plumage cycles exist for several species (Humphrey & Parkes 1959). Many species have two distinct plumages: the basic and the alternate, which are attained by the prebasic and prealternate molts, respectively (H-P nomenclature, Humphrey & Parkes 1959). Howell *et al.* (2003) reviewed this nomenclature and proposed a new terminology for the first plumage in the cycle (first basic plumage according H-P nomenclature), the formative plumage attained by preformative molt (first prebasic molt according H-P nomenclature). The occurrence and extent of prealternate molt in closely related species differs considerably (e.g. Lanyon 1975, Banks 1978) and New World blackbirds are not an exception. In particular, within the brood parasitic cowbirds (*Molothrus* spp., Icteridae) the occurrence of prealternate molt varies

between species ranging from zero, as in the Shiny Cowbird (*M. bonariensis*; Lowther & Post 1999) to frequent in all age-sex classes, as in the Bronzed Cowbird (*M. aeneus*; Lowther 1995). In all cases in which there is prealternate molt it is partial. Incomplete preformative molt is a common pattern in many passerines, therefore juveniles can be distinguished from adults in their first year of hatching (Pyle 1997).

The cowbirds encompass five obligate brood parasitic species (i.e. species that lay their eggs in nests of other species -hosts-, which provide all parental care for eggs and young) that show great variation in the number of hosts they use (Ortega 1998). The Screaming Cowbird (*M. rufoaxillaris*) is one of the more specialized brood parasites and uses almost exclusively one host, the Baywing (*Agelaioides badius*; Friedmann 1929, Fraga 1998, De Mársico *et al.* 2010) while the Shiny Cowbird (*M. bonariensis*) is an extreme generalist with more than 250 known hosts (Lowther 2011). Adult Screaming Cowbirds are considered sexually monochromatic (but see Cabezas *et al.* 2011). Both sexes are glossed black and, as its specific name suggests, they have a rufous axillary patch of feathers. A striking feature of this species is that young exhibit a plumage mimetic with that of Baywings, their main host (Hudson 1874, Fraga 1979) and they start the preformative molt at the time they became independent from their foster parents (Hudson 1874, Friedmann 1929). Adult Shiny Cowbirds are sexually dichromatic, males are lustrous black with iridescent and bluish gloss and female are grayish brown (Friedmann 1929, Lowther & Post 1999). Shiny Cowbird fledglings have female-like plumage, but noticeably streaked on the underparts (Friedmann 1929, Jaramillo & Burke 1999).

In this study we describe the timing and the extent of the preformative molt in Screaming and Shiny Cowbirds. To achieve this we established a small captive population of Screaming and Shiny Cowbirds that were

collected from host nests before fledging, hand-reared until nutritional independence and then kept in captivity until they completed their preformative molt.

MATERIALS AND METHODS

Study area. We collected nestlings of Screaming and Shiny Cowbirds during the breeding season 2009-2010 at "Reserva El Destino" (35°08' S, 57°23' W), in the province of Buenos Aires, Argentina. The study site is a flat area of 320 ha within the Biosphere Reserve "Parque Costero del Sur" (MAB-UNESCO). The area comprises a mosaic of marshy grasslands and woodland patches dominated by *Celtis tala* and *Scutia buxifolia*. Both cowbird species are resident in the area and breed from late September to mid January (Shiny Cowbirds) and from early December to late February (Screaming Cowbirds).

Capture and nurture of birds. We removed cowbird nestlings from host nests when they were 8-10 days of age, between late October 2009 and early February 2010. We collected 12 Screaming Cowbird nestlings from 12 nests of Baywings and 20 Shiny Cowbird nestlings from 12 nests of Chalk-browed Mockingbirds (*Mimus saturninus*), four nests of House Wrens (*Troglodytes aedon*), two nests of Rufous-collared Sparrows (*Zonotrichia capensis*), one nest of Baywings and one nest of Rufous Homeros (*Furnarius rufus*). After removal, we transported the young to the bioterium of the Faculty of Exact and Natural Sciences at the University of Buenos Aires. We fed young by hand every 1-2 hours from 6 AM to 8 PM with a mix of smashed potato, boiled egg, CeDé food for insectivorous birds and water until they were 30 days of age. We also provide them water *ad-libitum* through a plastic Pasteur pipette. As young growth, we gradually changed the diet to a mix of CeDé food for insectivorous birds, millet and chicken feed supplemented with larvae of *Zophobas morio* once a week. Water

was provided *ad-libitum*. Once a month they received an antic-occideal treatment. During the first 30 days they were housed in wire cages of 40 x 30 x 30 cm in groups of 3-4 birds per cage. After that, they were moved to an outdoor aviary of 3 x 2 x 2.5 m where they were kept until they completed the molt.

Data collection. We examined the young to determine the presence of new body and flight feathers and we took photographs of different body regions every 14 days, since they were fully feathered (12-14 days of age approximately) until they completed their molt. We analyzed 7 areas for body feathers: crown, throat, chest, belly, back-rump and under- and upper-wing coverts. For each area we scored molt from 0 to 4 depending on the percentage of new feathers: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100%. The sum of scores for each area was an index of the body feathers molt score and ranged from 0 (no molt) to 28 (molt completed). We scored the molt of flight feathers (primaries and secondaries) according to the stage of feather growth: 0 = old feathers, 1 = pin feather, 2 = open pin feather, 3 = until half grown feather, 4 = until $\frac{3}{4}$ grown feather, and 5 = fully grown feathers. The sum of molt scores for each primary on the wing ($n = 9$ primaries) was an index of the primary feathers molt score, which ranged from 0 (no molt) to 45 (molt completed). Similarly, we obtained the secondary feathers molt score. We also analyzed molt of rectrices, but we did not score it.

Sex determination. We determined the sex of Screaming Cowbird young by amplification of a size-different intron within the highly conserved chromo-helicase-DNA binding protein (CHD) gene located on the avian sex chromosomes (Ellegren 1996). We collected a 15-30 μ l blood sample with an 80 μ l heparinized capillary tube through brachial venipuncture with a G31 needle. We immediately mixed the blood with 0.5 ml of lysis buffer (100mM Tris pH 8, 10 mM NaCl, 100mM EDTA, 2% SDS) and stored it at room temperature until analysis.

We extracted DNA from blood samples using a standard salting-out protocol (Miller *et al.* 1988) and amplified the diagnostic sex-linked alleles using the P8/P2 primer set (Griffiths *et al.* 1998). We performed amplifications in 10 μ l reaction volumes using 50–100 ng of DNA template, 0.5 mM forward and reverse primers, 0.25 mM dNTPs, 2.5 mM MgCl₂, and 0.25 u Taq Polymerase. We set annealing temperatures at 50 °C and repeated for 30 cycles. We separated PCR products in 3% agarose gels stained with gel red. The presence of one band indicated males (ZZ), whereas two bands indicated females (ZW).

Statistical analysis. We used Mann-Whitney tests to examine differences between species and sexes. For statistical analyses, we used Statistica version 8.0 (StatSoft 2009). All *P*-values are two-tailed with *P* set at 0.05.

RESULTS

All Screaming and Shiny Cowbirds underwent a complete preformative molt and acquired the preformative plumage within six months of age (Fig. 1 and 3). There were no sexual differences in the age at which molt started for Screaming (Mann-Whitney test: $Z = -0.52$, $P = 0.71$) and Shiny (Mann-Whitney test: $Z = 1.85$, $P = 0.06$) Cowbirds. Also, we did not observe sexual differences in the temporal pattern of molt. Screaming Cowbirds started to molt before Shiny Cowbirds (median = 37 days of age, range: 27-49 days vs. median = 61 days of age, range: 32-85 days; Mann-Whitney test: $Z = 3.19$, $P < 0.01$, Fig. 1), but the age at which individuals acquired the formative plumage did not differ between species (Screaming Cowbird: median = 167 days, range: 154-180 days; Shiny Cowbird: median = 181 days of age, range: 152-214 days, Mann-Whitney test: $Z = 1.14$, $P = 0.09$; Fig 1).

In Screaming Cowbirds, juveniles did not show under-wing coverts, but this area was the first that acquired black feathers,

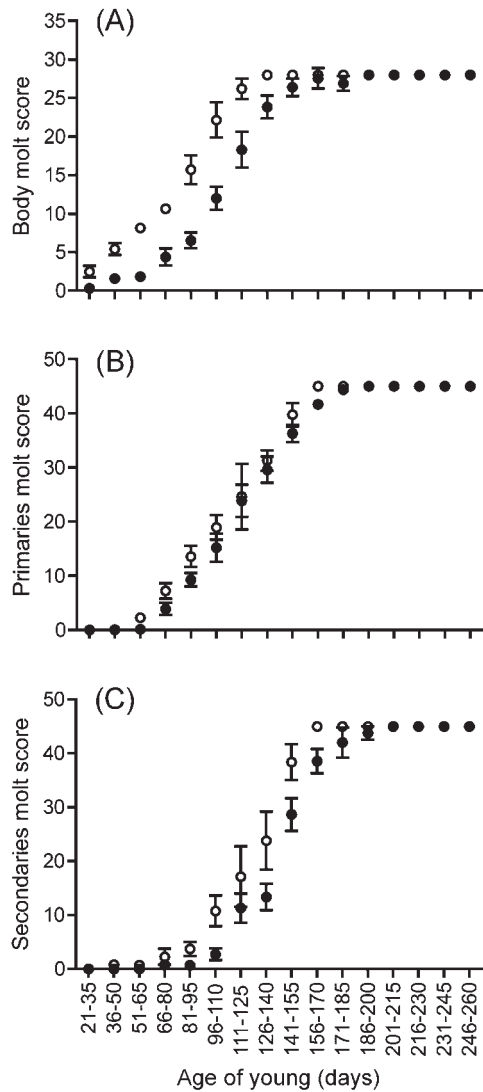


FIG. 1: Molt scores of (A) body, (B) primaries and (C) secondaries of Screaming (white circles) and Shiny Cowbirds (black circles) during prebasic molt.

like formative basic plumage (Fig. 2). After under-wing coverts initiated molting, all body showed black spots; beginning with chest and back-rump, followed by crown and throat, and lastly by upper-wing covers. The molt of flight feathers (Fig. 3A) started once the body

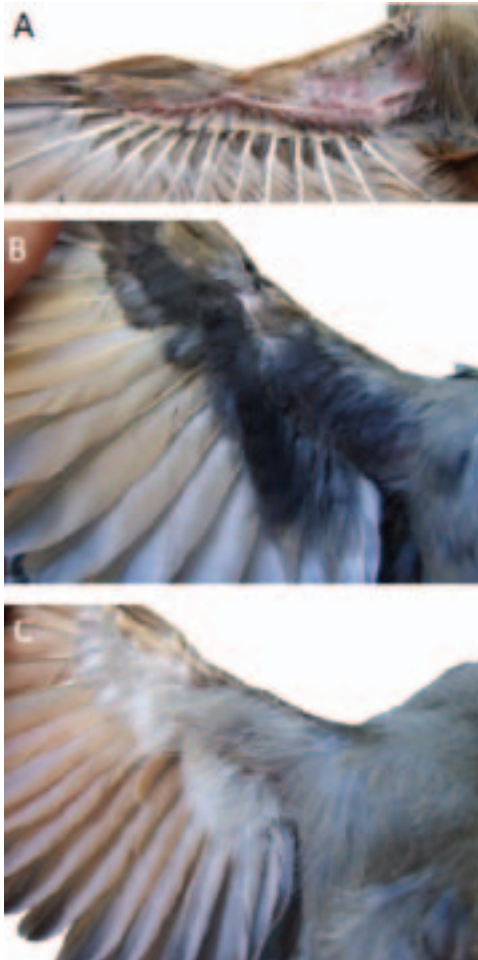


FIG. 2. View of Screaming Cowbirds underwing coverts at (A) 35 and (B) 52 days of age and view of Baywings underwing coverts at (C) 45 days of age.

molted 26-40% of the feathers (Figs. 1). The molt of primary feathers began at 57 days of age with P1 (range: 52-71 days, Fig. 1B) and finished with P9, while the molt of secondary feathers started from S1 to S5 and from S9 to S5 at the time primary 5 (P5) was molting (Fig. 3A).

In Shiny Cowbirds, molt began at the crown and in some cases at the chest and back-rump, followed by the throat, upper-wing coverts and

finally under-wing coverts. The molt of flight feathers started once the body molted 10-26% of the feathers (Fig 1). The molt of primary feathers started at 73 days of age (range: 62-94 days, Fig. 1B), while the molt of secondary feathers started at the time P4 and P5 were molting (Fig. 3B). The temporal pattern of the molt of flight feathers in Shiny Cowbird was similar to that of Screaming Cowbirds (Fig. 3). In both species the retrices were the last feathers that finished molt (Screaming Cowbirds = median: 148 days of age, range = 127-180 days; Shiny Cowbirds: 124 days of age, range: 123-214 days).

Juvenile plumage of Screaming Cowbirds was mimetic to that of Baywings except that Screaming Cowbird juveniles did not show black color in lores and around eyes (Fig. 4). Also under wing-coverts were black in Screaming Cowbirds (as the definitive plumage), but pale abelline gray in Baywings.

DISCUSSION

Our results show that the sequence and timing of molt in Screaming and Shiny Cowbirds present some similarities, as both species first molt body feathers, then flight feathers and finally tail feathers. Besides, in both species the sequence of molt of primary feathers starts with P1 and finishes with P9 and molt of secondary feathers starts with S1 and S9 and finishes with S5. Finally, both species have a complete preformative molt. However, we found differences between Shiny and Screaming Cowbirds in: 1) the age at which the molt starts and 2) the sequence at which they renew body feathers. Screaming Cowbirds began their preformative molt one month before Shiny Cowbirds and their molt began with the growth of under-wing black coverts, while in Shiny Cowbirds, it began with crown and chest feathers, and underwing coverts were the last body feathers that molted. The pattern of body and wing



FIG. 3. Sequence of first preformative molt. Photographs of (A) Screaming Cowbirds and (B) Shiny Cowbirds at different stage of plumage maturation (days of age).

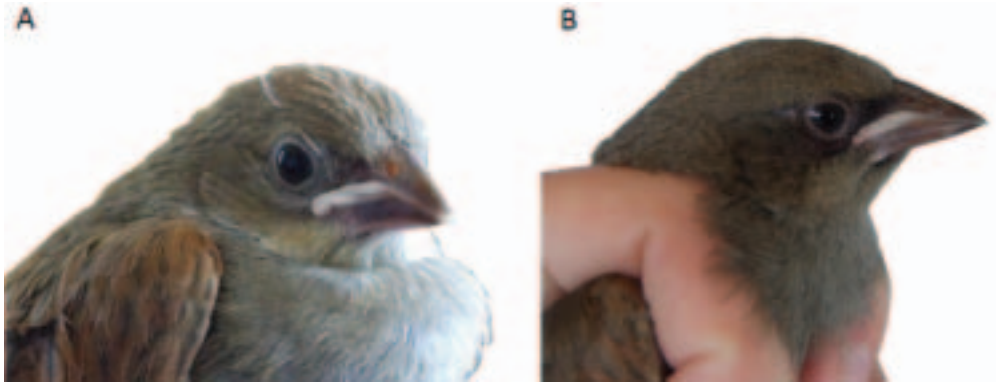


FIG 4. Photographs showing the difference in the black color in lores and around eyes between (A) Screaming Cowbirds and (B) Baywings.

molt that we observed in Screaming Cowbirds coincides with that described by Friedmann (1929), except that he did not mention the molt of under-wing coverts. Similarly, the pattern of molt observed in Shiny Cowbirds was similar to that described by Friedman (1929) and Pyle (1997), except that we did not observe juvenile feathers retained after the preformative molt, which indicates that in this population the preformative molt is complete, differing from other populations. However, we observed that some of the primary feathers of the formative plumage did not show the iridescence color like the definitive basic plumage. Therefore, individuals of one year could be distinguished from adults by primary feathers. Further studies are necessary to confirm this observation.

Body and flight feathers of Screaming Cowbird juveniles appear to be mimetic with those of its main host, the Baywing (Hudson 1874, Friedman 1929). However, we found that Screaming Cowbird juveniles did not show black color in lores and around eyes, and underwing coverts were black (Jaramillo & Burke 1999). These two features would allow researchers to easily distinguishing between young of both species. Baywing adults (parents and helpers) feed young of Screaming

Cowbirds until they became independent at 30-35 days of age (Fraga 1996, Ursino pers. observ.). Baywings also feed non-mimetic Shiny Cowbird young when they are in the nest (i.e. until they are 11-12 days of age). However, they stop feeding Shiny Cowbird young when they leave the nest (De Mársico *et al.* submitted). This suggests that Baywings use body and flight feather coloration (but not plumage color in lores and around eyes) as a cue to recognize their own young and provide parental care to them after they leave the nest. One hypothesis to explain why Screaming Cowbirds first start to molt under-wing coverts (and not body covers) is that to molt body coverts could negatively affect the parental care they receive from their foster parents. However, Screaming Cowbirds could start the molt after they are nutritionally independent (as Shiny Cowbirds do). Interestingly, at the time Screaming Cowbirds start molting, they join conspecifics flocks. One alternative hypothesis to explain the short latency in start the molt is that it would favor the acceptance of Screaming Cowbird young in conspecifics flocks (it would allow Screaming Cowbird adults to recognize juveniles and tolerate them). Further studies that analyze the interactions between young and adult cowbirds at the time

young join conspecifics flocks are necessary to test this hypothesis.

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