

The Wilson Journal of Ornithology 122(4):795–799, 2010

Screaming Cowbird Parasitism of Nests of Solitary Caciques and Cattle Tyrants

Alejandro G. Di Giacomo,¹ Bettina Mahler,² and Juan C. Reborada^{2,3}

ABSTRACT.—The Screaming Cowbird (*Molothrus rufoaxillaris*) is one of the most specialized brood parasites with only three known hosts: Baywing (*Agelaioides badius*), the main host throughout most of its range, and two alternative hosts in some areas of its distribution, Chopi Blackbird (*Gnorimopsar chopi*) and Brown-and-yellow Marshbird (*Pseudoleistes virescens*). We studied Screaming Cowbird parasitism in northeast Argentina where this parasite uses Baywings and Chopi Blackbirds as hosts. We monitored 69 nests of Baywings, 251 of Chopi Blackbirds, 31 of Solitary Caciques (*Cacicus solitarius*), and 30 of Cattle Tyrants (*Machetornis rixosa*). The frequency of Screaming Cowbird parasitism on Baywing nests was 80% and was 46% for Chopi Blackbirds. We recorded one event of Screaming Cowbird parasitism on one nest of Solitary Caciques and three events of Screaming Cowbird parasitism on one nest of Cattle Tyrants. The identities of parasitic eggs in both hosts were confirmed by sequencing the mtDNA control region. We propose

these events of parasitism resulted from recognition errors by Screaming Cowbird females that regularly parasitize Baywings and Chopi Blackbirds. The nest of Solitary Caciques had been frequently visited by a pair of Baywings before Screaming Cowbird parasitism occurred, and the nest of Cattle Tyrants was near an active Chopi Blackbird nest that had been previously parasitized by Screaming Cowbirds. *Received 5 January 2010. Accepted 7 April 2010.*

The Screaming Cowbird (*Molothrus rufoaxillaris*) is a specialized brood parasite, which exclusively uses the Baywing (*Agelaioides badius*) as a host throughout most of its range (Mason 1980, Fraga 1998). It also parasitizes the Brown-and-yellow Marshbird (*Pseudoleistes virescens*) in some areas (Mermoz and Fernández 2003) and the Chopi Blackbird (*Gnorimopsar chopi*) (Sick 1985, Di Giacomo 2005). Other species have been reported as hosts of the Screaming Cowbird, but there is general agreement that most of these reports were based on mis-identified Shiny Cowbird (*M. bonariensis*) eggs (Friedmann 1963, Mason 1980, Fraga 1986).

One hypothesis posited to explain host specificity in brood parasites is that females imprint on

¹Departamento de Conservación, Aves Argentinas-Asociación Ornitológica del Plata, Matheu 1246, C1249AAB Buenos Aires, Argentina.

²Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II Ciudad Universitaria, C1428EGA Buenos Aires, Argentina.

³Corresponding author;
e-mail: reborada@ege.fcen.uba.ar

their foster parents and, once mature, search for nests of the same species in which to lay their eggs (Nicolai 1964, Slagsvold and Hansen 2001). This hypothesis has been directly supported by experiments with captive brood-parasitic Village Indigobirds (*Vidua chalybeata*) bred in captivity and foster-reared by their normal host or by an experimental foster species and tested as adults for host choice (Payne et al. 2000). Indirect evidence in support of this hypothesis include: (1) the host specificity of Common Cuckoo (*Cuculus canorus*) females, but not males (Marchetti et al. 1998), and (2) the association between host species and parasite's mitochondrial, but not nuclear DNA (Gibbs et al. 2000).

Host imprinting and occurrence of recognition errors when parasitic females search for host nests (i.e., to lay eggs in nests of hosts other than the foster parents) may result in use of new hosts. This may lead to the formation of host specific races, if only females imprint on their hosts as by Common Cuckoos (Gibbs et al. 2000) or in speciation, if both males and females imprint on their hosts (i.e., indigobirds [*Vidua* spp.]; Payne et al. 2002, Sorenson et al. 2003).

Friedmann and Kiff (1985) suggested host imprinting may also occur in cowbirds and individual females may be host specialists. The evidence, however, is controversial for Brown-headed Cowbirds (*M. ater*). One study showed no genetic differentiation in mtDNA between cowbird chicks raised by two different hosts (Gibbs et al. 1997), whereas results from another study at the same site, showed that half of the females laid in nests of a single host (Alderson et al. 1999). MtDNA haplotype frequency distributions in Shiny Cowbirds differed among parasitic chicks from nests of four hosts (Mahler et al. 2007), which indicate the occurrence of a non-random laying behavior by females of this parasite. A recent study (Mahler et al. 2009) of the host specialist Screaming Cowbird, showed that frequency distributions of mtDNA haplotypes differed between cowbird chicks from nests of Baywings and Chopi Blackbirds. This result indicates host choice by females is not random and female Screaming Cowbirds may preferentially parasitize nests of the foster parents. The number of mutations between directly related haplotypes in the latter study was small, which indicates that haplotype divergence in the population is relatively recent in evolutionary terms. Mahler et al. (2009) suggested a few Screaming

Cowbirds may have switched recently from Baywings to Chopi Blackbirds, giving rise to a new race that only uses the latter host.

Successful host switches require females to lay eggs in nests of a host other than the foster parent (recognition errors), that the new host successfully rears parasitic females, and these females subsequently use the new foster parent as a host. We present direct evidence through genotyped eggs of Screaming Cowbird parasitism of two new hosts: Solitary Cacique (*Cacicus solitarius*) and Cattle Tyrant (*Machetornis rixosa*). Our study was conducted in an area where Screaming Cowbirds parasitize Baywings and Chopi Blackbirds. We propose parasitism of the new hosts resulted from recognition errors by Screaming Cowbird females that regularly parasitize Baywings and Chopi Blackbirds.

METHODS

Study Area.—The study was conducted at 'Reserva Ecológica El Bagual' in the Province of Formosa, Argentina (26° 18' 17.5" S; 58° 49' 51.1" W). This reserve is an open savanna of 3,300 ha in the eastern or humid Chaco region. Average annual rainfall at the study site is 1,350 mm and mean monthly temperatures vary from 16.9° C in July to 26.7° C in January.

Data Collection and Analysis.—We monitored nests of potential hosts of Shiny and Screaming cowbirds during the 1997–1998 to 2008–2009 breeding seasons. We found 69 nests of Baywings, 251 of Chopi Blackbirds, 31 of Solitary Caciues, and 30 of Cattle Tyrants. Most nests were found during construction, laying, and incubation, and were visited every 2–3 days until chicks fledged or the nest failed. Individual eggs were marked with waterproof ink and assigned to the host or to Shiny or Screaming cowbirds on the basis of background color, spotting pattern, and shape (Fraga 1983). We also genetically identified 27 and 31 Screaming Cowbird eggs in nests of Baywings and Chopi Blackbirds, respectively, by sequencing the control region of the mtDNA (Mahler et al. 2009). Screaming Cowbird eggs from nests of Solitary Caciues and Cattle Tyrants were also genetically identified. The initial assignment of Screaming Cowbird eggs on the basis of color and spotting pattern was confirmed by genetic analysis in all cases. Frequency of parasitism was calculated as number of nests with parasite eggs or chicks divided by total number of

nests found, and intensity of parasitism as number of parasitic eggs per parasitized nest.

Genetic Identification of Screaming Cowbird Eggs.—We sequenced a 600 base pair fragment of the mtDNA control region using primers MBO-L1 and MBO-H2 (Mahler et al. 2007). We collected Screaming Cowbird eggs and artificially incubated them for 48 hrs to obtain some embryonic development. We kept eggs at -20° C until they were processed. Embryonic tissue was obtained from the eggs and stored in DMSO buffer for posterior DNA extraction following a standard ethanol protocol (Miller et al. 1988). PCR reactions were performed as described by Mahler et al. (2009). We sequenced the amplified products on an Applied Biosystems Model 3100 Genetic Analyzer using ABI Big DyeTM Terminator Chemistry. We compiled the sequences in Bioedit Version 7.0.5.3 software (Hall 1999) and compared them with those deposited in the EMBL, GenBank, under accession numbers EU199785-EU199795 (Mahler et al. 2009).

RESULTS

Screaming Cowbirds regularly parasitized Baywings and Chopi Blackbirds at our study site. The frequency of parasitism of Baywing nests was 80% ($n = 69$ nests) with an intensity of parasitism of 2.4 ± 0.2 eggs per parasitized nest ($\bar{x} \pm SE$, $n = 51$ nests). Frequency and intensity of parasitism of Chopi Blackbird nests were 46% ($n = 251$ nests) and 3.0 ± 0.2 eggs ($n = 116$ nests), respectively.

Shiny Cowbirds parasitized only one of 31 nests of the Solitary Cacique (3%). We recorded one event of Screaming Cowbird parasitism in one nest of Solitary Caciues in December 2007. Baywings had visited this nest several times during the previous days and had tried, unsuccessfully, to usurp the nest. Screaming Cowbird parasitism occurred before host laying and, after parasitism, the caciues deserted the nest. The egg was collected and incubated, DNA extracted, and the control region sequenced. The sequence of the mtDNA control region corresponded to Screaming Cowbirds' haplotype H5 (Mahler et al. 2009).

We also monitored 30 nests of Cattle Tyrants but Shiny Cowbirds parasitized none of them. We recorded three events of Screaming Cowbird parasitism of one Cattle Tyrant nest during laying in November 2008. This nest was <1 m from an active Chopi Blackbird nest that was also multiple parasitized by Screaming Cowbirds. Cattle Ty-

rants incubated the Screaming Cowbird's eggs of which one hatched but the chick disappeared from the nest 48–72 hrs after hatching. We collected the other two eggs, one of which was rotten and we could not extract DNA from it. The sequence of the mtDNA control region of the other egg corresponded to Screaming Cowbirds' haplotype H1 (Mahler et al. 2009).

DISCUSSION

We confirmed Screaming Cowbird parasitism of two new hosts, Solitary Cacique and Cattle Tyrant, in an area where this parasite regularly uses Baywings and Chopi Blackbirds. Solitary Caciues and Cattle Tyrants were previously reported as parasitized by Shiny Cowbirds, but until now they had not been mentioned as parasitized by Screaming Cowbirds (Ortega 1998). We propose these parasitism events resulted from recognition errors by female Screaming Cowbirds that regularly parasitize Baywings and Chopi Blackbirds. A pair of Baywings had frequently visited the nest of Solitary Caciues before Screaming Cowbird parasitism occurred, and the nest of the Cattle Tyrants was near an active nest of Chopi Blackbirds that had been previously parasitized by Screaming Cowbirds.

Baywings seldom build nests but rather exploit a wide variety of covered nesting sites, including old nests built by other species and holes in trees (Fraga 1988, De Mársico et al. 2010). Baywings at our study site usually use old nests of Greater Thornbirds (*Phacellodomus ruber*) and Little Thornbirds (*P. sibilatrix*) but, at times they usurp and use nests of Solitary Caciues and becards (*Pachyramphus* spp.) (Di Giacomo 2005). This may promote recognition errors by Screaming Cowbird females searching for nests occupied by its main host. Baywings also use holes in trees, which may have favored colonization of hole-nesting Chopi Blackbirds.

The first step in the process of colonization of a new host would be occurrence of recognition errors when parasitic females search for host nests (i.e., to lay eggs in nests of a host species other than their foster parents). We show this type of error may occur at low frequencies in Screaming Cowbirds. However, the low frequency of parasitism of Solitary Caciues and Cattle Tyrants also suggests the other steps necessary for parasitism of a new host (i.e., rearing parasitic females that subsequently use the same species as

a host; Payne et al. 2000, 2002; Sorenson et al. 2003) have not yet occurred in these hosts. The three known hosts of Screaming Cowbirds (Baywings, Chopti Blackbirds, and Brown-and-yellow Marshbirds) are cooperative breeders (Orians et al. 1977, Fraga 1991, Di Giacomo 2005), which suggests competition for food with nest mates is critical for Screaming Cowbirds. Thus, this parasite has only been successful in hosts' nests where competition is less intense, as breeding pairs have one or more helpers at the nest that contribute to chick provisioning (De Mársico and Reboresda 2008). This hypothesis requires further investigation.

ACKNOWLEDGMENTS

We thank Alparamis SA and Aves Argentinas/Asociación Ornitológica del Plata for allowing us to conduct this study at Reserva El Bagual. We thank Peter Lowther and Spencer Sealy for helpful comments on a previous version of this manuscript. BM and JCR are Research Fellows of CONICET. This study was supported by Agencia Nacional de Promoción Científica y Tecnológica (Grant 06-00215) and the University of Buenos Aires (Grant X184).

LITERATURE CITED

- ALDERSON, G. W., H. L. GIBBS, AND S. G. SEALY. 1999. Determining the reproductive behaviour of individual Brown-headed Cowbirds using microsatellite DNA markers. *Animal Behaviour* 58:895–905.
- DE MÁRSICO, M. C. AND J. C. REBORESDA. 2008. Differential reproductive success favours strong host preferences in a highly specialized brood parasite. *Proceedings of the Royal Society, Series B* 275:2499–2506.
- DE MÁRSICO, M. C., B. MAHLER, AND J. C. REBORESDA. 2010. Reproductive success and nestling growth of Baywings parasitized by Screaming and Shiny cowbirds. *Wilson Journal of Ornithology* 122:417–431.
- DI GIACOMO, A. G. 2005. Aves de la Reserva El Bagual. Pages 201–465 in *Historia natural y paisaje de la Reserva El Bagual, Provincia de Formosa, Argentina* (A. G. Di Giacomo and S. F. Krapovickas, Editors). *Temas de Naturaleza y Conservación* 4. Aves Argentinas/Asociación Ornitológica del Plata, Buenos Aires.
- FRAGA, R. M. 1983. The eggs of the parasitic Screaming Cowbird (*Molothrus rufoaxillaris*) and its host, the Bay-winged Cowbird (*M. badius*): is there evidence for mimicry? *Journal für Ornithologie* 124:187–193.
- FRAGA, R. M. 1986. The Bay-winged Cowbird (*Molothrus badius*) and its brood parasites: interactions, coevolution and comparative efficiency. Thesis. University of California at Santa Barbara, California, USA.
- FRAGA, R. M. 1991. Nest sites and breeding success of Bay-winged Cowbirds (*Molothrus badius*). *Journal für Ornithologie* 129:175–183.
- FRAGA, R. M. 1991. The social system of a communal breeder, the Bay-winged Cowbird *Molothrus badius*. *Ethology* 89:195–210.
- FRAGA, R. M. 1998. Interactions of the parasitic Screaming and Shiny cowbirds (*Molothrus rufoaxillaris* and *M. bonariensis*) with a shared host, the Bay-winged Cowbird (*Molothrus badius*). Pages 173–193 in *Parasitic cowbirds and their hosts. Studies in coevolution* (S. I. Rothstein and S. K. Robinson, Editors). Oxford University Press, New York, USA.
- FRIEDMANN, H. 1963. Host relations of the parasitic cowbirds. U.S. National Museum Bulletin 233.
- FRIEDMANN, H. AND L. F. KIFF. 1985. The parasitic cowbirds and their hosts. *Proceedings of the Western Foundation of Vertebrate Zoology* 2:225–304.
- GIBBS, H. L., P. MILLER, G. ALDERSON, AND S. G. SEALY. 1997. Genetic analysis of Brown-headed Cowbirds *Molothrus ater* raised by different hosts: data from mtDNA and microsatellite DNA markers. *Molecular Ecology* 6:189–193.
- GIBBS, H. L., K. MARCHETTI, M. D. SORENSON, M. DE L. BROOKE, N. B. DAVIES, AND H. NAKAMURA. 2000. Genetic evidence for female host-specific races of the Common Cuckoo. *Nature* 407:183–186.
- HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.
- MAHLER, B., V. A. CONFALONIERI, I. J. LOVETTE, AND J. C. REBORESDA. 2007. Partial host fidelity in nest selection by the Shiny Cowbird (*Molothrus bonariensis*), a highly generalist avian brood parasite. *Journal of Evolutionary Biology* 20:1918–1923.
- MAHLER, B., Y. SARQUIS ADAMSON, A. G. DI GIACOMO, V. A. CONFALONIERI, AND J. C. REBORESDA. 2009. Utilization of a new host in the host-specialist brood parasite *Molothrus rufoaxillaris*: host switch or host acquisition? *Behavioral Ecology and Sociobiology* 63:1603–1608.
- MARCHETTI, K., H. L. GIBBS, AND H. NAKAMURA. 1998. Host-race formation in the Common Cuckoo. *Science* 282:471–472.
- MASON, P. 1980. Ecological and evolutionary aspects of host selection in cowbirds. Thesis. University of Texas, Austin, USA.
- MERMOZ, M. E. AND G. J. FERNÁNDEZ. 2003. Breeding success of a specialist brood parasite, the Screaming Cowbird, parasitizing an alternative host. *Condor* 105:63–72.
- MILLER, S. A., D. D. DYKES, AND H. F. POLESKY. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 16:1215.
- NICOLAI, J. 1964. Der Brutparasitismus der Viduinae als ethologisches problem. Prägnanzphänomene als faktoren der rassen- und artbildung. *Zeitschrift für Tierpsychologie* 21:129–204.
- ORIAN, G. H., C. E. ORIAN, AND K. J. ORIAN. 1977. Helpers at the nest in some Argentine blackbirds. Pages 137–151 in *Evolutionary ecology* (B. Stonehouse and C. Perrins, Editors). Macmillan Press, London, United Kingdom.

- ORTEGA, C. P. 1998. Cowbirds and other brood parasites. University of Arizona Press, Tucson, USA.
- PAYNE, R. B., L. L. PAYNE, J. L. WOODS, AND M. D. SORENSON. 2000. Imprinting and the origin of parasite-host species associations in brood-parasitic indigobirds, *Vidua chalybeata*. *Animal Behaviour* 59:69–81.
- PAYNE, R., B. K. HUSTLER, R. STJERNSTEDT, K. M. SEFC, AND M. D. SORENSON. 2002. Behavioural and genetic evidence of a recent population switch to a novel host species in brood-parasitic indigobirds *Vidua chalybeata*. *Ibis* 144:373–383.
- SICK, H. 1985. Ornitologia brasileira: uma introdução. University of Brasilia, Brazil.
- SLAGSVOLD, T. AND B. T. HANSEN. 2001. Sexual imprinting and the origin of obligate brood parasitism in birds. *American Naturalist* 158:354–367.
- SORENSON, M. D., K. M. SEFC, AND R. B. PAYNE. 2003. Speciation by host switch in brood parasitic indigobirds. *Nature* 424:928–931.