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Partial host fidelity in nest selection by the shiny cowbird (*Molothrus bonariensis*), a highly generalist avian brood parasite

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Abstract

Obligate avian brood parasites can be host specialists or host generalists. In turn, individual females within generalist brood parasites may themselves be host specialists or generalists. The shiny cowbird *Molothrus bonariensis* is an extreme generalist, but little is known about individual female host fidelity. We examined variation in mitochondrial control region sequences from cowbird chicks found in nests of four common Argentinean hosts. Haplotype frequency distributions differed among cowbird chicks from nests of these hosts, primarily because eggs laid in nests of house wrens *Troglodytes aedon* differed genetically from those laid in nests of the other three hosts (chalk-browed mockingbird *Mimus saturninus*, brown-and-yellow marshbird *Pseudoleistes virescens*, and rufous-collared sparrow *Zonotrichia capensis*). These differences in a maternally inherited marker indicate the presence of a nonrandom laying behaviour in the females of this otherwise generalist brood parasite, which may be guided by choice for nest type, as house wrens nest in cavities whereas the other three species are open cup nesters.

Introduction

Obligate avian brood parasites lay their eggs in nests of other host species, which thereafter provide all parental care. Brood parasites may be host specialists, if they use one or a few host species, or host generalists, if they parasitize many hosts. Coevolutionary interactions between parasites and hosts result in an evolutionary arms race (Davies *et al.*, 1989; Davies & Brooke, 1989; Rothstein, 1990; Rothstein & Robinson, 1998; Davies, 2000), in which generalist brood parasites may evolve two different strategies: (1) they may become specialists at an individual level, with each female consistently parasitizing one particular host species, and eventually forming host-specific lineages that mimic the eggs of the host (Brooke & Davies, 1988; Avilés & Møller, 2004; Starling *et al.*, 2006); or (2) they may become generalists, with individual females parasitizing several host species using a shotgun strategy in which eggs are nonmimetic and the use of a great number of hosts assures that at least some of the eggs are not rejected (Kattan, 1997; Rothstein & Robinson, 1998). Host specificity in both males and females may lead to host-linked population divergence and speciation (Sorenson *et al.*, 2003).

Previous studies have found contrasting laying strategies in females of two generalist brood parasites. In the common cuckoo *Cuculus canorus*, each female specializes on one particular host species (Marchetti *et al.*, 1998; Gibbs *et al.*, 2000; Skjelseth *et al.*, 2004) or the most common species in one particular habitat (Teuschl *et al.*, 1998; Honza *et al.*, 2001), and this behaviour has led to female host-specific races (gentes), with female lineages laying mimetic eggs that resemble those of the host they parasitize (Brooke & Davies, 1988; Moksnes & Røskraft, 1995). In the brown-headed cowbird *Molothrus ater*, females of the same population have been found to use both specialist and generalist laying strategies (Alderson *et al.*, 1999; Woolfenden *et al.*, 2003; Strausberger & Ashley, 2005), with a consistent nest site

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selection pattern (Hauber, 2001; Hoover *et al.*, 2006). When assayed via maternally inherited mitochondrial DNA (mtDNA) markers, these laying patterns produced either mtDNA differentiation among chicks raised by different hosts (Gibbs *et al.*, 2000; but see Gibbs *et al.*, 1996) or no differentiation among chicks found in different host nests (Gibbs *et al.*, 1997), respectively. In common cuckoos, the correlation of mtDNA and host use could be mediated by an imprinting process, in which a female inherits the mtDNA from her mother and also shares her choice of host species (Gibbs *et al.*, 2000). Rarely, a female will lay in a host nest different from the one she was raised in, thus originating a host switch in her daughters, and giving rise to a new gens (Davies, 2000). This host-switching mechanism stemming from errors in the recognition of the host has also led to colonization of new hosts and speciation in host-specialist *Vidua* finches (Payne *et al.*, 2002; Sorenson *et al.*, 2003).

The shiny cowbird *Molothrus bonariensis* is an extreme generalist brood parasite that uses more than 200 species as hosts (Friedmann & Kiff, 1985; Ortega, 1998). However, there is no information about whether this generalism at a population level is based on specialist or generalist individual females. Although attempts have been made to study the laying pattern of shiny cowbirds in particular host species (Kattan, 1997; Lyon, 1997; Mermoz & Reboreda, 1999), no study has focused on more than one host, as it is very difficult to follow female shiny cowbirds because they do not defend territories and are highly mobile. A recent study on a shiny cowbird population in Puerto Rico that has been subject to a control programme has suggested host specificity in shiny cowbird females' laying behaviour (López-Ortiz *et al.*, 2006).

The aim of our study was to test whether there are female shiny cowbird lineages specialized on one particular host, using a rapidly evolving molecular marker, the mtDNA control region. We determined control region haplotype distributions among cowbird chicks found in nests of four different hosts in Buenos Aires Province, Argentina. The four host species studied are all successful hosts of the shiny cowbird, and in our study area they all have high levels of parasitism: 66% of nests in the chalk-browed mockingbird *Mimus saturninus* (Fiorini & Reboreda, 2006), 60% in the house wren *Troglodytes aedon* (Tuero *et al.*, 2007), 67% in the brown-and-yellow marshbird *Pseudoleistes virescens* (Mermoz & Reboreda, 1999) and 69% in the rufous-collared sparrow *Zonotrichia capensis* (Fraga, 1978).

We expected to find genetic differences among chicks raised by different hosts if individual females are host specialists and if female chicks raised in the nest of a particular host have a strong tendency to parasitize that same host as adults, whereas we expected no pattern of genetic differentiation if females are host generalists or differ in host use with their mothers. In doing so, we assume that host fidelity has a detectable effect on the genetic structure of cowbird populations. This can be achieved in only several generations (Rothstein, 1975). We found a difference in haplotype frequency distributions among cowbird chicks found in house wren nests and those found in nests of the other three hosts. This nonrandom laying behaviour in shiny cowbird females may be guided by choice for nest type, as house wrens are cavity nesters and the other three species are open cup nesters.

Material and methods

Cowbird samples

Samples were collected from cowbird eggs or nestlings found in nests of four host species at three different locations in Buenos Aires Province, Argentina, during three cowbird breeding seasons (October–January 2002–2003, 2003–2004 and 2004–2005; Table 1). The three sampling locations correspond to study sites used for related research projects and are between 70 and 150 km from each other: Magdalena (35°08′S, 57°23′W), General Lavalle (36°26′S, 56°25′W) and Chascomús (35°34′S, 58°01′W). Samples were collected from chalk-browed mockingbird ($n = 30$), brown-and-yellow marshbird ($n = 25$) and rufous-collared sparrow ($n = 17$) nests found in the study areas, and from wooden nest boxes placed in the three locations that were used by house wrens ($n = 29$).

Cowbird genetic samples were obtained from host nests either as eggs or as blood taken from nestlings. Freshly laid eggs were artificially incubated to obtain some embryonic development prior to DNA extraction, and eggs found with some degree of incubation were directly processed. In the reproductive season 2004–2005, in which nest searching was specifically carried out to collect cowbird egg samples, we took photographs of the eggs, using standardized lighting conditions, to record eggshell colour patterns before dissection. Embryonic tissue was extracted from the eggs and stored in DMSO buffer for posterior genetic analyses. Blood samples were taken via wing venipuncture of nestlings and stored in lysis buffer.

Table 1 Number of shiny cowbird samples by host species and sample location for three breeding seasons (October–January 2002–2003, 2003–2004 and 2004–2005).

Host species location	Magdalena	General Lavalle	Chascomús	Total
House wren	22	2	5	29
Chalk-browed mockingbird	28	1	1	30
Brown-and-yellow marshbird	0	25	0	25
Rufous-collared sparrow	0	17	0	17
Total	50	45	6	101

mtDNA analyses

To assess mtDNA variation, we sequenced a 1120-base pair fragment of the control region, using two sets of primers. One set of primers, GSH-12 s and GSL-GLU, has been used before on brown-headed cowbirds (Gibbs *et al.*, 1997). We developed another set of primers to amplify the left-hand domain of the control region: MBO-L1 (5′-CAGTACGTTTTCTTCTTTATTTCCAGG-3′) and MBO-H2 (5′-TGAGGGGTTTATTGAAGAGACGC-3′). DNA was extracted from blood and tissue samples with Eppendorf (Hamburg, Germany) and QIAGEN (Hilden, Germany) extraction kits. PCR amplifications for both sets of primers were performed in 10-μl reaction volumes using 50–100 ng of DNA template, 0.5 μM forward and reverse primers, 0.25 μM dNTPs, 2.5 mM $MgCl_2$ and 0.25 u Taq-Polymerase Sigma (St. Louis, MO, USA) Jumpstart Taq. Annealing temperatures were set at 50 °C and repeated for 30 cycles. Amplified products were sequenced on an Applied Biosystems (Foster City, CA, USA) Model 3100 Genetic Analyzer using ABI Big Dye™ Terminator Chemistry. Nucleotide sequences have been deposited in the EMBL, GenBank, under accession numbers DQ683547–DQ683561.

Data analysis

The sequences were compiled in Sequencher v.3.1.1 (Genecodes Corp., Ann Arbor, MI, USA) and Bioedit v.7.0.5.3 software (Hall, 1999), and aligned using Clustal W (Thompson *et al.*, 1994). To control for unintentional amplification of nuclear pseudogenes (Sorenson & Fleischer, 1996), sequences were checked carefully for double peaks, and more than half of the DNA samples were extracted from embryonic tissue, where the ratio of mitochondrial : nuclear genomes is many times higher than in avian erythrocytes (which retain their nuclei but generally lack functional mitochondria after maturation), thus decreasing the likelihood of amplifying nuclear pseudogenes. Embryonic samples and blood samples yielded the same haplotypes. Phylogenetic relationships among mtDNA haplotypes were inferred using maximum parsimony, as implemented in TNT (Goloboff *et al.*, 2003). Exact searches were performed using the 'implicit enumeration' option.

The program Arlequin v.2.0 (Schneider *et al.*, 2000) was used to test for population structure based on the frequencies of haplotypes among hosts and sampling locations. Genetic differentiation among host species and sampling locations was assessed using AMOVA (Excoffier *et al.*, 1992), which partitions total variance into within- vs. between-group components (Hudson *et al.*, 1992), through Φ_{ST} that takes into account both haplotype frequencies and molecular pairwise differences. The average number of nucleotide differences between sequences was estimated using the Kimura two-parameter model of nucleotide substitution. Significance levels were determined using permutation procedures as implemented in Arlequin.

Statistical analyses were performed on all samples, and were repeated controlling for different factors that could bias our results: (1) sampling location; (2) multiple samples of the same female; and (3) host egg rejection behaviour. (1) We compared haplotype frequency distributions among the three localities and haplotype frequency distributions between host species of the same locality. (2) We incorporated only the offspring that were almost certainly derived from different females. As individual females lay eggs with a consistent eggshell colouration (Fleischer, 1985; Dufty, 1994; Lyon, 1997), for each locality and season we excluded all but one sample found in the same host that shared a similar eggshell colour pattern (i.e. background colour, spot colour, spot size and distribution of spots on the eggshell) and presented the same haplotype. Eggs laid on the same day were considered to belong to different females, irrespective of their colouration. (3) We repeated the analysis including only spotted eggs. Shiny cowbird eggs can be white immaculate or spotted (Hudson, 1920; Mason, 1986) and hosts respond differently to the presence of shiny cowbird eggs in their nests: some of them accept all egg morphs, others reject all egg morphs and still others accept only spotted eggs. The hosts included in this study vary in their egg rejection behaviour, and whereas the chalk-browed mockingbird and the brown-and-yellow marshbird reject white eggs, this egg morph is accepted by the house wren and the rufous-collared sparrow. Therefore, we controlled for a bias in haplotype frequency distribution related to the presence of white eggs.

Results

A 1120-bp segment of the mtDNA control region was sequenced from 101 cowbird eggs or nestlings from nests of four different hosts. A total of 17 nucleotide sites varied among samples resulting in 15 different haplotypes. We found three frequent haplotypes (H1, H2 and H7), four less-frequent haplotypes (H4, H5, H6 and H11) and eight rare haplotypes (Fig. 1).

Phylogenetic relationships among the different haplotypes yielded four most parsimonious networks. Figure 1 shows a network representing one of these unrooted trees, whose topology differed from the rest in the position of a few connections.

Haplotype frequency distributions between cowbird chicks found in nests of different hosts were nonrandom ($\Phi_{ST} = 0.12$; $P < 0.001$). Specifically, chicks found in nests of house wrens differed genetically from chicks of the other three hosts (pairwise Φ_{ST} values = 0.20–0.23; $P < 0.001$), which in turn did not differ between each other (pairwise Φ_{ST} values = −0.03–0.04; $P > 0.05$). These patterns did not change when analyses were restricted to the smaller subset of samples that excluded offspring that could have been mothered by the same

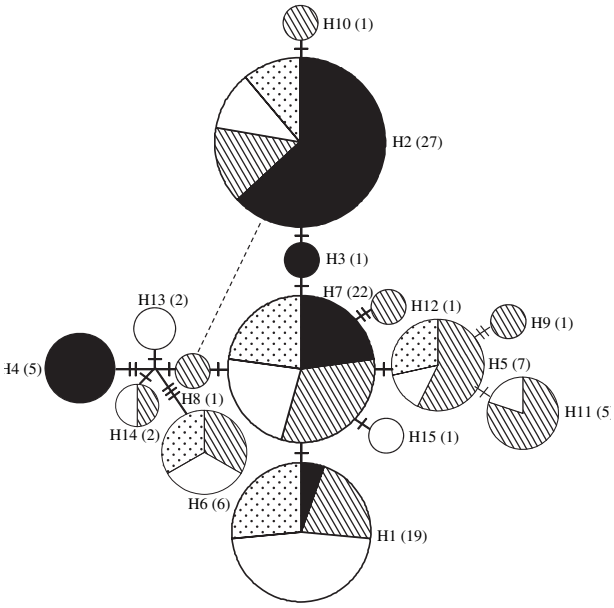

Fig. 1 Unrooted maximum parsimony network for 15 shiny cowbird haplotypes (H1–H15). Numbers in parentheses show the number of sampled eggs/chicks per haplotype. Circle size is proportional to haplotype frequency and hatchmarks show the number of nucleotide differences between observed haplotypes. Shading indicates the proportion of each of four host species associated with each cowbird haplotype (black: house wren; lined: chalk-browed mockingbird; white: brown-and-yellow marshbird; dotted: rufous-collared sparrow). Alternative connections defining other equally parsimonious trees are shown by dotted lines.

Table 2 Haplotype distribution of cowbird samples collected from host nests in Magdalena and General Lavalle.

	Magdalena		General Lavalle	
Haplotype	House wren	Chalk-browed mockingbird	Brown-and-yellow marshbird	Rufous-collared sparrow
H1	0	4	9	5
H2	14	4	3	3
H3	0	0	0	0
H4	5	0	0	0
H5	0	4	1	2
H6	0	2	2	2
H7	3	5	5	5
H8	0	1	0	0
H9	0	1	0	0
H10	0	1	0	0
H11	0	4	1	0
H12	0	1	0	0
H13	0	0	2	0
H14	0	1	1	0
H15	0	0	1	0

female ($n = 80$; $\Phi_{ST} = 0.09$ across all hosts, $P = 0.001$), or to only spotted eggs ($n = 87$; Φ_{ST} across all hosts = 0.08; $P < 0.001$).

Cowbird haplotype frequency distributions differed significantly among sampling locations ($\Phi_{ST} = 0.06$; $P < 0.01$). However, this pattern proved to be a consequence of cowbird chicks sampled from house wren nests, which occurred predominately at the Magdalena location (Table 1). When the analysis was repeated excluding all house wren nest samples, there were no differences in haplotype frequencies among locations ($\Phi_{ST} = 0.01$; $P = 0.2$). Additionally, the analysis of the samples collected in Magdalena showed that haplotype frequency distributions differed between cowbird chicks found in chalk-browed mockingbird and house wren nests ($\Phi_{ST} = 0.23$, $P < 0.001$; Table 2). On the contrary, there were no differences between cowbird chicks of brown-and-yellow marshbird and rufous-collared sparrow nests in General Lavalle ($\Phi_{ST} = -0.03$, $P > 0.5$; Table 2).

Discussion

Our study showed that the population of shiny cowbird females that lay in nests of house wrens is genetically differentiated from the population that uses the other three hosts studied, suggesting that host selection is not random in this species.

Several hypotheses have been proposed to explain how female brood parasites find specific hosts: (a) host imprinting (Brooke & Davies, 1991; Payne *et al.*, 1998, 2000), in which the female nestling learns the characteristics of her foster parents before leaving the nest; (b) natal philopatry (Brooke & Davies, 1991), in which the female returns to the area where she was born and chooses hosts randomly; (c) nest site choice (Moksnes & Røskraft, 1995), in which the female chooses a group of host species with similar eggs and nest sites, and searches for nests at random within this group; and (d) habitat imprinting (Teuschl *et al.*, 1998; Vogl *et al.*, 2002), in which female chicks learn the characteristics of the habitat where they grew up by an imprinting process and choose similar habitats later for laying eggs. However, these hypotheses fail to explain the host-use patterns found in shiny cowbirds: (a) shiny cowbird female lineages were not host specific for all hosts studied; (b) hosts were not chosen randomly in one area, as nests of house wrens and rufous-collared sparrows or chalk-browed mockingbirds may be found in the same areas and are often only a few metres apart, but were not used indiscriminately; (c) although nest site and eggs of the house wren differ from the other three hosts, the latter also differ between each other in nest site and egg characteristics; and (d) shiny cowbird chicks reared in marshes (brown-and-yellow marshbirds) and low trees or bushes of grasslands (chalk-brown mockingbirds) do not differ in mitochondrial haplotype frequencies.

What other factors drive these partially nonrandom laying patterns in our shiny cowbird population? Although chalk-browed mockingbirds and brown-and-yellow marshbirds are relatively large birds that

weigh approximately 75 g, and house wrens are small birds that weigh less than 13 g, differences in body size between hosts cannot explain the observed laying pattern. The rufous-collared sparrow has an intermediate size (18 g), but with a weight much closer to that of house wrens than to the other two hosts. Host selection by body size would predict that females that use the nests of house wrens also parasitize rufous-collared sparrows. Instead we found that rufous-collared sparrows are used as hosts by females of the same haplotypes that use both large hosts.

Our results suggest that host choice by shiny cowbird females may be explained by nest-type characteristics. Although built in different nest sites, the three hosts that are used by genetically similar females have open cup nests, whereas the house wren is a cavity nester. We propose that chicks that are reared in a particular type of nest are imprinted with that type such that their later search image for laying targets that general nest type. Kattan (1997) studied the laying behaviour of shiny cowbirds in the house wren in Colombia, and noticed that although nests of another successful host, the pied water-tyrant *Fluvicola pica* (Cruz & Andrews, 1997) were available, these were not used by the females. Interestingly, both hosts have closed nests, but whereas the house wren uses cavities, the pied water-tyrant builds an oval ball of grasses and other plant material. His results are in accordance with our hypothesis, provided that shiny cowbirds are able to distinguish between both types of closed nests. Alternatively, shiny cowbird females raised in house wren nests may preferentially parasitize house wrens as adults owing to imprinting on other aspects of wren behaviour or ecology.

In contrast to the pattern found by Gibbs *et al.* (2000) in common cuckoos, shiny cowbirds of a given haplotype were found in the nests of different host species (Fig. 1, Table 2). This pattern can be explained in two ways. First, there may be imperfect nest selection in cowbird females, and although the females of each haplotype primarily parasitize hosts of a particular nest type, they may occasionally deposit their eggs in different nests, with these 'mistakes' occurring more frequently than in common cuckoos. The haplotype frequency pattern found here is consistent with this explanation, as H1 and H7 females use almost entirely open cup nests, whereas H2 females mainly use cavity nests (Fig. 1). Second, change in host use may be so rapid that it is not tracked by the mtDNA marker used in this study. Although the use of a new host species may evolve in the course of few generations, we were able to detect differences in mtDNA haplotype frequency distribution in our study population. We do not think that the lack of differentiation among the three hosts that use open cup nests is a consequence of more recent host specialization, because we should see specialization for any of these three hosts in at least some of the haplotypes. Only haplotype 11 is found largely in chalk-browed mockingbirds nests (Fig. 1), but this is most likely to be explained by a sampling artefact. Alternatively, the lack of mtDNA differentiation may be explained by more frequent host switches among species that build open cup nests, thus masking differences in host use between females. We cannot discard this possibility, but it would still show a stronger association of shiny cowbirds to house wrens than to the other three hosts.

In conclusion, we found that laying patterns of shiny cowbird females in host nests are nonrandom. Our results suggest that host fidelity may be guided by nest characteristics, with females selecting for a particular type of nest to lay their eggs, and that switches to other nest types are frequent in this species.

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References

Alderson, G.W., Gibbs, H.L. & Sealy, S.G. 1999. Determining the reproductive behaviour of individual brown-headed cowbirds using microsatellite DNA markers. *Anim. Behav.* **58**: 895–905.

Avilés, J.M. & Møller, A.P. 2004. How is host egg mimicry maintained in the cuckoo (*Cuculus canorus*)? *Biol. J. Linn. Soc.* **82**: 57–68.

Brooke, M. de L. & Davies, N.B. 1988. Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature* **335**: 630–632.

Brooke, M. de L. & Davies, N.B. 1991. A failure to demonstrate host imprinting in the cuckoo (*Cuculus canorus*) and alternative hypotheses for the maintenance of egg mimicry. *Ethology* **89**: 154–166.

Cruz, A. & Andrews, R.W. 1997. The breeding biology of the Pied Water–Tyrant and its interactions with the shiny cowbird in Venezuela. *J. Field Ornithol.* **68**: 91–97.

Davies, N.B. 2000. *Cuckoos, Cowbirds and Other Cheats*. T & A D Poyser, London.

Davies, N.B. & Brooke, M. de L. 1989. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. *J. Anim. Ecol.* **58**: 225–236.

Davies, N.B., Bourke, A.F.G. & Brooke, M. de L. 1989. Cuckoos and parasitic ants: interspecific brood parasitism as an evolutionary arms race. *Trends Ecol. Evol.* **4**: 274–278.

Dufty, A.M. Jr 1994. Rejection of foreign eggs by yellow-headed Blackbirds. *Condor* **96**: 799–801.

Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA

haplotypes: application to human mitochondrial DNA. *Genetics* **131**: 479–491.

Fiorini, V.D. & Reboreda, J.C. 2006. Cues used by shiny cowbirds (*Molothrus bonariensis*) to locate and parasitise chalk-browed mockingbird (*Mimus saturninus*) nests. *Behav. Ecol. Sociobiol.* **60**: 379–385.

Fleischer, R.C. 1985. A new technique to identify and assess the dispersion of eggs of individual brood parasites. *Behav. Ecol. Sociobiol.* **17**: 91–100.

Fraga, R.M. 1978. The rufous-collared sparrow as a host of the shiny cowbird. *Wilson Bull.* **90**: 271–284.

Friedmann, H. & Kiff, L.F. 1985. The parasitic cowbirds and their hosts. *Proc. West. Found. Vert. Zool.* **2**: 225–302.

Gibbs, H.L., Brooke, M. de L. & Davies, N.B. 1996. Analysis of genetic differentiation of host races of the common cuckoo *Cuculus canorus* using mitochondrial and microsatellite DNA variation. *Proc. R. Soc. B* **263**: 89–96.

Gibbs, H.L., Miller, P., Alderson, G. & Sealy, S.G. 1997. Genetic analysis of brown-headed cowbirds *Molothrus ater* raised by different hosts: Data from mtDNA and microsatellite DNA markers. *Mol. Ecol.* **6**: 189–193.

Gibbs, H.L., Marchetti, K., Sorenson, M.D., Brooke, M. de L., Davies, N.B. & Nakamura, H. 2000. Genetic evidence for female host-specific races of the common cuckoo. *Nature* **407**: 183–186.

Goloboff, P., Farris, J. & Nixon, K. 2003. TNT: Tree Analysis Using New Technology: Program and documentation available from the authors. Available at: http://www.zmuc.dk/public/phylogeny/tnt.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**: 95–98.

Hauber, M.E. 2001. Site selection and repeatability in brown-headed cowbird (*Molothrus ater*) parasitism of eastern phoebe (*Sayornis phoebe*) nests. *Can. J. Zool.* **79**: 1518–1523.

Honza, M., Moksnes, A., Roskaft, E. & Stokke, B.G. 2001. How are different common cuckoo *Cuculus canorus* egg morphs maintained? An evaluation of different hypotheses. *Ardea* **89**: 341–352.

Hoover, J.P., Yasukawa, K. & Hauber, M.E. 2006. Spatially and temporally structured avian brood parasitism affects the fitness benefits of hosts' rejection strategies. *Anim. Behav.* **72**: 881–890.

Hudson, W.H. 1920. *Birds of La Plata*. J. M. Dent, New York.

Hudson, R., Slatkin, M. & Maddison, W. 1992. Estimation of levels of gene flow from DNA sequence data. *Genetics* **132**: 583–589.

Kattan, G.H. 1997. Shiny cowbirds follow the 'shotgun' strategy of brood parasitism. *Anim. Behav.* **53**: 647–654.

López-Ortiz, R., Ventosa-Febles, E.A., Ramos-Alvarez, K.R., Medina-Miranda, R. & Cruz, A. 2006. Reduction in host use suggests host specificity in individual shiny cowbirds (*Molothrus bonariensis*). *Ornitol. Neotrop.* **17**: 259–269.

Lyon, B.E. 1997. Spatial patterns of shiny cowbird brood parasitism on chestnut-capped blackbirds. *Anim. Behav.* **54**: 927–939.

Marchetti, K., Gibbs, H.L. & Nakamura, H. 1998. Host-race formation in the common cuckoo. *Science* **282**: 471–472.

Mason, P. 1986. Brood parasitism in a host generalist, the shiny cowbird. II. Host selection. *Auk* **103**: 61–69.

Mermoz, M.E. & Reboreda, J.C. 1999. Egg-laying behaviour by shiny cowbirds parasitizing brown-and-yellow marshbirds. *Anim. Behav.* **58**: 873–882.

Moksnes, A. & Røskraft, E. 1995. Egg-morphs and host preference in the common cuckoo (*Cuculus canorus*): an analysis of cuckoo and host eggs from European museum collections. *J. Zool.* **236**: 625–648.

Ortega, C. 1998. *Cowbirds and Other Brood Parasites*. University of Arizona Press, Tucson, AZ.

Payne, R.B., Payne, L.L. & Woods, J.L. 1998. Song learning in brood-parasitic indigobirds *Vidua chalybeata*: song mimicry of the host species. *Anim. Behav.* **55**: 1537–1553.

Payne, R.B., Payne, L.L., Woods, J.L. & Sorenson, M.D. 2000. Imprinting and the origin of parasite–host species associations in brood-parasitic indigobirds, *Vidua chalybeata*. *Anim. Behav.* **59**: 69–81.

Payne, R.B., Hustler, K., Stjernstedt, R., Sefc, K.M. & Sorenson, M.D. 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.

Rothstein, S.I. 1975. Evolutionary rates and host defenses against avian brood parasitism. *Am. Nat.* **109**: 161–176.

Rothstein, S.I. 1990. A model system for coevolution: avian brood parasitism. *Annu. Rev. Ecol. Syst.* **21**: 481–508.

Rothstein, S.I. & Robinson, S.K. 1998. *Parasitic Birds and their Hosts*. Oxford University Press, Oxford.

Schneider, S., Roessli, D. & Excoffier, L. 2000. *Arlequin ver. 2.000: A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Skjelseth, S., Moksnes, A., Røskaft, E., Kleven, O., Gibbs, H.L., Taborsky, M., Taborsky, B. & Honza, M. 2004. Parentage and host preference in the common cuckoo *Cuculus canorus*. *J. Avian Biol.* **35**: 21–24.

Sorenson, M.D. & Fleischer, M.D. 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proc. Natl Acad. Sci. U.S.A.* **93**: 15239–15243.

Sorenson, M.D., Sefc, K.M. & Payne, R.B. 2003. Speciation by host switch in brood parasitic indigobirds. *Nature* **424**: 928–931.

Starling, M., Heinsohn, R., Cockburn, A. & Langmore, N.E. 2006. Cryptic gentes revealed in pallid cuckoos *Cuculus pallidus* using reflectance spectrophotometry. *Proc. R. Soc. B* **273**: 1929–1934.

Strausberger, B.M. & Ashley, M.V. 2005. Host use strategies of individual female brown-headed cowbirds *Molothrus ater* in a diverse avian community. *J. Avian Biol.* **36**: 313–321.

Teuschl, Y., Taborsky, B. & Taborsky, M. 1998. How do cuckoos find their hosts? The role of habitat imprinting. *Anim. Behav.* **56**: 1425–1433.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680.

Tuero, D.T., Fiorini, V.D. & Reboreda, J.C. 2007. Effects of shiny cowbird parasitism on different components of house wren reproductive success. *Ibis*, doi:10.1111/j.1474-919x.2007.00676.x.

Vogl, W., Taborsky, B., Teuschl, Y., Taborsky, M. & Honza, M. 2002. Cuckoo females preferentially use specific habitats when searching for host nests. *Anim. Behav.* **64**: 843–850.

Woolfenden, B.E., Gibbs, H.L., Sealy, S.G. & McMaster, D.G. 2003. Host use and fecundity of individual female brown-headed cowbirds. *Anim. Behav.* **66**: 95–106.

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