

## Behavioural and genetic evidence of a recent population switch to a novel host species in brood-parasitic indigobirds *Vidua chalybeata*

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We describe a population of Village Indigobirds *Vidua chalybeata* on the Zambezi River that parasitizes the nests and mimics the songs of a novel host species, Brown Firefinch *Lagonosticta nitidula*, yet coexists with a population that mimics the usual host species of this indigobird, Red-billed Firefinch *L. senegala*. Male indigobirds mimicking the song of *L. nitidula* are morphologically indistinguishable from those that mimic songs of the usual host, *L. senegala*. Likewise, nestling indigobirds in broods of *L. nitidula* and *L. senegala* are similar in having mouth markings that mimic the nestlings of *L. senegala* rather than those of the novel host. Molecular genetic evidence indicates that the host switch to *L. nitidula* has involved at least four different indigobird matrilineal lineages. Indigobirds that are associated with *L. nitidula* are genetically similar to the indigobirds associated with sympatric *L. senegala*, and not to the indigobirds associated with west African Bar-breasted Firefinch *L. rufopicta*, the species that is most closely related to *L. nitidula*. Because field and experimental studies show that female indigobirds prefer males that mimic the songs of their own foster species, and females choose the same host to parasitize, the indigobirds reared by a novel host form a distinct breeding population. Taken together, behavioural, morphological and genetic evidence indicates a recent host switch by indigobirds in the Zambezi region from their old host *L. senegala* to a new host *L. nitidula*.

African indigobirds *Vidua* spp. are the most host-specific of the brood-parasitic birds, as each species is generally associated with a single species of estrildid finch host. Nestling indigobirds mimic the mouth colours and patterns of the nestlings of their respective host species, and this nestling mimicry may aid in gaining parental care. Adult male indigobirds mimic host calls and songs, and this song mimicry identifies the host species that reared him as a young bird under parental care. The male's song mimicry may therefore advertise his genetic quality to a female brood parasite that was reared by the same host species (Nicolai 1964, Payne 1997). In contrast to brood-parasitic cuckoos in which females of one species mimic the eggs of different hosts (Davies & Brooke 1998, Gibbs *et al.* 2000), the indigobirds

that mimic different hosts in most cases are morphologically and behaviourally distinct species.

The species-specific associations between parasitic indigobirds and their hosts may be ancient, if the parasites diverged along with their hosts in a process of parallel speciation or cospeciation (Nicolai 1964), or they may be more recent, if the different parasitic species evolved after switching from established hosts to novel host species (Payne 1973, 1997). Similarly, species-specific nestling mimicry may derive from gradual coevolutionary change as host and parasitic lineages diverged in parallel, or nestling mimicry may have evolved in each parasitic species after a successful host switch and subsequent selection to match the young of the new host.

These historical hypotheses (ancient cospeciation, Nicolai 1964, Page 1994, Siddall 1996; recent host switches, Payne 1997) can be tested by comparing the phylogenies and genetic distances of hosts and

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parasites. The ancient cospeciation hypothesis predicts that the indigobirds which parasitize sister species of hosts should be each others' closest phylogenetic relatives. It also predicts that the genetic distances between the respective species in the host clade and the parasite clade should be nearly equal (the physiology and generation times are similar in these small finches). In contrast, if indigobirds have recently colonized a novel host species because they were successful in a behavioural switch to the novel host, then the indigobirds that parasitize different host species within the same geographical region should be more closely related to other indigobirds within the same region than they are to indigobirds that parasitize the same host species in another region. Also, the genetic distances between indigobird species should be less than between their corresponding host species (Klein *et al.* 1993, Klein & Payne 1998). Another prediction of the recent host switch hypothesis is that some indigobird populations are associated with a host species other than the one that they have evolved to mimic. This idea is consistent with the behaviour of one indigobird species in west Africa, the Fonio Indigobird *Vidua camerunensis*, which mimics the songs of five species of estrildid finches (African Firefinch *Lagonosticta rubricata*, Mali Firefinch *L. virata*, Blackbellied Firefinch *L. rara*, Brown Twinspot *Clytospiza monteiri*, Dybowski's Twinspot *Euschistospiza dybowskii*), although the directions of host switches in this species are unknown (Payne & Payne 1994, 1995, Klein & Payne 1998, R.B. Payne, pers. obs.).

We describe a population of Village Indigobirds *Vidua chalybeata* on the Zambezi River that parasitizes the nests and mimics the songs of a novel finch host species. This population provides direct behavioural and molecular genetic evidence that indigobirds have been successful in switching from one host species to another under natural conditions.

## STUDY AREA AND METHODS

### Survey area

Fieldwork was carried out from 1996 to 2000 on the upper Zambezi River in Zimbabwe and Zambia. Most birds were on a mid-channel island near Kazungula (17°45'S, 25°16'E), 60 km upstream from Victoria Falls. The island was 3 km × 400 m, ringed by trees on the outer bank with reedbeds in the centre and downstream edges. Field localities in

Zambia also included Katombora rapids (17°45'S, 25°23'E) and Mosi-oa-Tunya National Park (17°50'S, 25°46'E) 14 km above the falls. Other observations were made in Zambia at Mambove upstream from Kazungula, at Tongabezi and Sindabezi island between Katombora and Mosi-oa-Tunya, and in Lochinvar National Park (15°57'S, 27°15'E), and in Zimbabwe from Imbabala Lodge to the Botswana border at Kazungula, and elsewhere (Payne *et al.* 1993, Klein & Payne 1998). In addition, observations, song recordings and genetic samples were obtained in Gambia, Nigeria and Cameroon (Payne 1973, 1982, Payne 1998, Klein & Payne 1998).

### Songs and captures

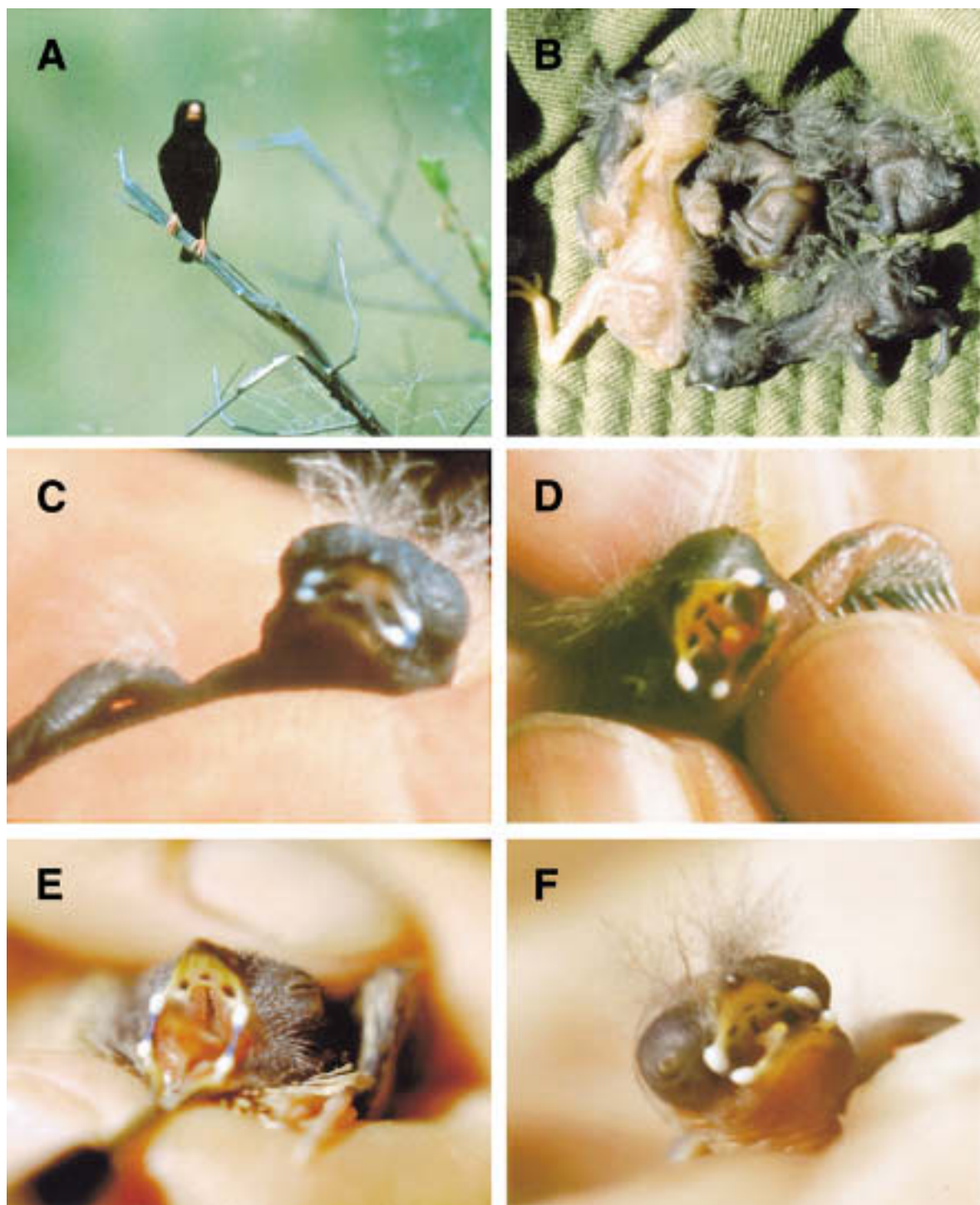
Songs of indigobirds and firefinches were recorded with a Sony TC-D5M cassette tape recorder and 33-cm parabolic reflector. Birds were colour-ringed and released for observation or were collected as study specimens. Kay 5500-DSP audiospectrograms of songs were compared with songs of local estrildid species.

Recorded songs were used to attract each indigobird male to a mist net. A female was netted when she visited a male at his call-site where they mated (Payne 1973, Payne & Payne 1977). Indigobirds were measured (wing), and colours of bill, feet and breeding plumage were matched to samples in a colour reference (Kornerup & Wanscher 1967).

For molecular genetic analysis we sampled indigobirds that were associated with *L. senegala* and *L. nitidula* firefinches in the upper Zambezi region as well as other *V. chalybeata* in southern Africa (Zambia, Zimbabwe and Malawi) and west Africa (Gambia, Nigeria and Cameroon). We also sampled Wilson's Indigobird *Vidua wilsoni* in Nigeria and Cameroon. *V. wilsoni* is the species-specific brood parasite and song mimic of Bar-breasted Firefinch *L. rufopicta*, which is the west African counterpart of *L. nitidula* and is sometimes considered the same species (Payne 1982). *V. wilsoni* is morphologically distinct from other indigobirds; males are small and have purple plumage and pale wings, and nestlings mimic the mouth pattern of *L. rufopicta*, which has a continuous white and bluish gape flange rather than gape papillae as in *L. nitidula* (Payne 1982, Payne & Payne 1994, Fig. 1C).

### Genetic analyses

Samples for genetic analysis included one of the following: (1) a feather was pulled from the wing or the



**Figure 1.** (A) Male *Vidua chalybeata* with mimetic songs of *Lagonosticta nitidula*; (B) nestlings in a mixed-species brood of *V. chalybeata* and *L. nitidula* with dissimilar skin and tarsus colours (pale in *V. chalybeata*, black in *L. nitidula*); (C) black skin and pinkish mouth of nestling *L. nitidula*; (D) pale skin and yellow mouth of nestling *V. chalybeata* in nest of *L. nitidula*; (E) pale skin and yellow mouth of nestling *L. senegala*; (F) pale skin and yellow mouth of nestling *Vidua chalybeata*. Birds (A–D) were on the island in the Zambezi, (E) was at Lochinvar National Park and (F) was bred in captivity of *V. c. amauropteryx* parents; it developed into a male similar in appearance to (A).

tail and stored dry in a small envelope; (2) a small (~10 µL) blood sample obtained by venipuncture of the tarsal vein was collected in a heparinized micro-hematocrit capillary tube and stored in lysis buffer; (3) muscle tissue was stored in DMSO/EDTA buffer (Seutin *et al.* 1991).

Genomic DNA was isolated using a QIAquick Tissue Kit (QIAGEN). For feather samples, 30 µL of 100 mg/mL dithiothreitol was added to the digestion buffer (Cooper 1994). Two overlapping fragments comprising about 1100 base pairs (bp) and including most of the mitochondrial NADH dehydrogenase subunit 6 (ND6) gene, tRNA-glutamine, and the 5' half of the control region were PCR amplified and sequenced with primers pairs L16225 (Sorenson *et al.* 1999) and Finch5PR2 (5'-CATTTCAGTRAMTGTCTGATGGGGC-3'), and IndigoC1F1 (5'-TCTTCATGCTTTACAGGGTATG-3') and FinchC1R1 (5'-GGTATGGTCCTGAAGTTACAAC-3') for 80 indigobirds, 2–4 individuals of each host species, and appropriate outgroup taxa. For the host species and other estrildids, primer FireC1F1 (5'-TTTTCTHNTGACTTTTAGGGTATG-3') was used in place of IndigoC1F1. An additional 549 bp comprising half of the mitochondrial ND2 gene and part of tRNA-tryptophan was sequenced for the host species using primers L5758 and H6313 (Sorenson *et al.* 1999). PCR products were gel-purified in 1.5% low-melt agarose, excised from the gel, and recovered with a Gel Extraction Kit (QIAGEN). Double-stranded PCR products were sequenced directly with Taq DNA Polymerase FS (Applied Biosystems). Reaction products were run on an Applied Biosystems ABI 377 automated DNA sequencer.

Phylogenetic analysis of the sequence data was completed in PAUP\*, version 4.0b4 (Swofford 2000), using replicate heuristic searches with random addition of taxa to find the most parsimonious tree(s) with all characters and changes given equal weight. Gaps were treated as a fifth character state. Kimura 2-parameter genetic distances between clades (Kimura 1980, Steel *et al.* 1996) were based on sequences common to both the host and the parasite data sets and excluded positions with alignment gaps in one or more taxa.

To estimate kinship within a local breeding population of indigobirds, we also genotyped each indigobird for 11 nuclear microsatellite loci developed specifically for indigobirds (Indigo7, 8, 15, 27, 28, 29, 30, 37, 38, 40, 41; Sefc *et al.* 2001). Each locus was amplified in a 10-µL PCR reaction including ~30 ng DNA, 10 mM Tris-HCl pH 8.3, 50 mM KCl,

2.5 mM MgCl<sub>2</sub>, 125 M of each dNTP, 0.5 M of each primer and 0.25 U AmpliTaq DNA polymerase (Applied Biosystems). An annealing temperature of 52 °C was used for all loci except Indigo 37, for which we used 54 °C. Forward primers were labelled with a fluorescent dye (either 6-FAM, HEX or TET) allowing the length of amplified microsatellite alleles to be determined using the ABI 377 automated DNA sequencer and GeneScan software. Each lane on our microsatellite gels included internal size standards (GS-500 TAMRA) plus up to three loci from a given individual, each labelled with a different dye.

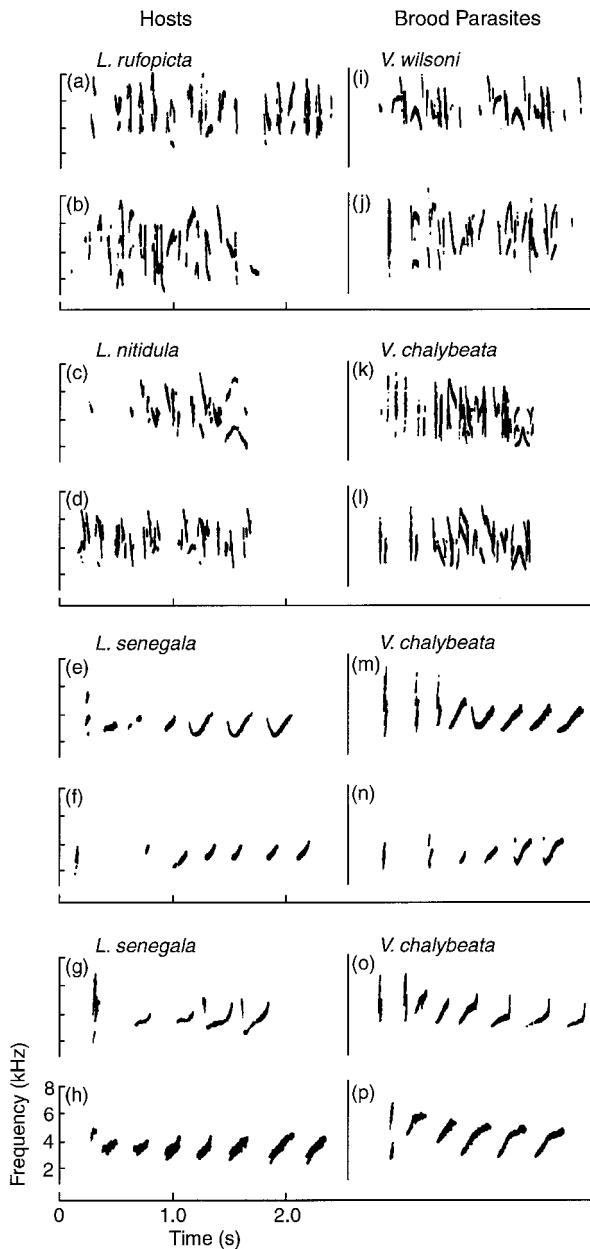
Using the program IDENTITY (Wagner & Sefc 1999), we screened all possible combinations of individuals among the sampled indigobirds to find trios with microsatellite genotypes consistent with those of an offspring and its two parents or pairs of individuals that could be parent and offspring. We also used KINSHIP (Queller & Goodnight 1989) to identify pairs of individuals that could be related at the level of full sibs among the *V. chalybeata* we sampled from southern Africa. The ratio of likelihoods for two individuals either being full sibs or being unrelated was compared to a null distribution of likelihood ratios derived from 10 001 simulated pairs of individuals based on allele frequencies in the total sample of southern *V. chalybeata*. Given the large number of pairwise comparisons made, we considered only those cases in which the significance level was less than 0.001 to be indicative of close relationship.

## RESULTS

In populations of village indigobirds *Vidua chalybeata* on Kazungula island, at Katombora and at Mosi-oa-Tunya, five males mimicked songs of Brown Firefinch *Lagonosticta nitidula*, the firefinch that was newly discovered as a host species. In the same localities and on the upper Zambezi at Mambove and Tongabezi, other males mimicked songs of the indigobirds' normal host, the Red-billed Firefinch *L. senegala*. The two firefinch species, *L. nitidula* and *L. senegala*, occur together in open woodland and grasslands near reedbeds. The indigobird nestlings and adults that are associated with *L. nitidula* are morphologically indistinguishable from the indigobirds that mimic *L. senegala*. The *V. chalybeata* that are associated with *L. nitidula* have a song and a host that is unusual for their species.

## Song mimicry

Songs of *L. nitidula* consist of a series of short, jingling or twittering notes, with most sound energy at 4–8 kHz (Payne 1982, Fig. 2c,d). A song has 9–11



**Figure 2.** Audiospectrograms of songs of host species *Lagonosticta rufopicta*, *L. nitidula*, *L. senegala*, and mimicry songs of their local brood parasites *Vidua wilsoni* and *V. chalybeata*. a, Taboru, Nigeria; b, Zaria, Nigeria; c, d, k, l, o, Kazungula island, Zambia; e, k, Garoua, Cameroon; f, j, Bukuru, Nigeria; g, h, Lochinvar National Park, Zambia; i, Tchébova, Cameroon; l, Dumbi, Nigeria.

notes per second, given in a jumbled, irregular pattern of high metallic notes and low nasal notes. Most notes descend in pitch, while some notes rise then fall to form a hat shape with a peak at 5 kHz and often with an overtone. The song has interspersed alarm notes, and it often ends with a descending whistle. Songs are as long as 4 s and are repeated with the same sequence of notes; a bird has two song types that differ in notes and their sequence. The social contact call is a short rising and falling note given singly or repeated; in the field a pair that was disturbed by us separated, then one gave the call and its mate came to it. In addition, *L. nitidula* gives an alarm call 'tik' that is a single note, abrupt, rising then falling, less than 0.04 s in duration. The call is given singly, in pairs or in short chatters at a rate of four notes per 0.3 s, when alarmed or disturbed, and often repeated in rapid succession in a regular sharp chatter, 12–18 per s, 'trrrittit tritritit'. The songs and calls of west African *L. rufopicta* are nearly identical (Payne 1982, Fig. 2a,b) except the long chattered version of alarm call was not heard in *L. rufopicta*.

In contrast, the songs of *L. senegala* consist of a harsh note (the alarm call, 'chick') followed by 2–6 whistles (contact calls, 'pea'), usually rising in pitch, 'chick, pea, pea ...' at rate of 3–6 notes per second (Fig. 2e–h). A male usually has an individualistic song theme, given repeatedly sometimes with the last few 'pea' notes lacking, and he sometimes has a second song theme. The contact call is a single or double slurred whistle 'pea' note. The alarm or excitement call is a short 'chick', given singly, or repeated in excitement as when a snake is near (Nicolai 1964, Morel 1973, Payne 1973, 1990, Payne *et al.* 1993, 1998, 2000). Songs vary regionally across Africa but are consistent in the 'chick-pea' pattern.

Most red-billed male indigobirds *V. chalybeata* in the upper Zambezi region mimicked calls and songs of their host species, *L. senegala*. Within 200 km of the region we recorded more than 100 males with these songs, including 87 males in earlier years at Lochinvar National Park in Zambia (Payne 1985, Payne *et al.* 1993). Songs of host finches and brood-parasite indigobirds were similar in the upper Zambezi, and they also were similar in west Africa (Fig. 2m–p). On Kazungula island, at Mosi-oa-Tunya and at Katombora, other male indigobirds were recorded with the mimicry calls and songs of *L. nitidula* rather than *L. senegala* (Fig. 2k,l).

In February 1996 three males on the island were heard with songs of *L. nitidula*, and three others (one with white bill and red feet) had songs of *L. senegala*.

Each male's call-site was within 0.4 km of another male, and each was in an African mangosteen *Garcinia livingstonei* tree. Birds were tape recorded, captured, and feathers sampled on 6 April, though birds were not ringed in 1996. In 1997 males appeared on the same call-sites, including two males that mimicked *L. nitidula*. In 1998, five males were colour-ringed for observation. The three that mimicked songs of *L. nitidula* were seen to visit each other's call-sites. All males on the island with songs of *L. nitidula* had identical mimicry songs as well as identical non-mimicry songs, much as in *V. chalybeata* males in Lochinvar National Park that mimicked *L. senegala* (Payne 1985).

At Mosi-oa-Tunya, the first male with songs of *L. nitidula* was recorded on 20 March 1996. On the same call-site, a male (perhaps the same one) was colour-ringed in 1997 and was seen and tape recorded there again in 1998. Six other males, all with songs of *L. senegala*, were recorded and colour-ringed in the park in 1997; one was observed repeatedly as late as 30 June 2000.

At Katombora all six males seen in 1997 were song mimics of *L. senegala*, but in 2000 one male was recorded with songs of *L. nitidula*, as were three with songs of *L. senegala*. The male with songs of *L. nitidula* was seen on the same call-site from 7 February through 30 June.

*L. nitidula* were common on Kazungula island and Katombora; they were not found in Mosi-oa-Tunya which was intensely grazed, but they were common on the upper Zambezi islands, within sight of the indigobird song mimic of *L. nitidula*. The details of indigobird song mimicry differed between these localities. The microgeographical variation in song suggests the occurrence of several song populations of indigobird *L. nitidula* mimics in the region.

## Morphology

Indigobirds *V. chalybeata* that mimic songs of *L. nitidula* in the upper Zambezi region have the same appearance as males that mimic songs of *L. senegala*, with steel-blue body plumage, brown wings, and red bill and feet (Fig. 1A), except for two males that mimicked *L. senegala* and had a white bill, one on the island and one in Mosi-oa-Tunya. Both red-billed and the two white-billed birds mimicked songs of *L. senegala*, as did white-billed indigobirds in northern Botswana (Payne 1973). Red-billed male *V. chalybeata* that mimicked song of *L. nitidula* had wings 63.8–69 mm ( $n = 6$ , mean =  $66.18 \pm 1.96$ ); red-

billed males that mimicked *L. senegala* in southern Zambia and Zimbabwe had wings 64–69 mm ( $n = 15$ , mean =  $65.87 \pm 1.60$ ), with complete overlap and no significant difference in mean size. These red-billed indigobirds in the Zambezi region were like those elsewhere in the range of the southern African subspecies *V. chalybeata amauropteryx* (Payne 1973). Also in the Zambezi region were males of another species, Purple Indigobird *V. purpurascens*, with white bills, whitish feet and song mimicry of Jameson's Firefinch *L. rhodopareia*.

The female captured and colour-ringed at the call-site of a male song mimic of *L. nitidula* and later identified as the mother of an indigobird nestling in a *L. nitidula* nest (see below) had a pink bill and feet, similar to female red-billed female *V. c. amauropteryx* throughout their range (Payne 1973, Payne & Payne 1977).

## Brood parasitism

On the island we found several nests of *L. nitidula* that were parasitized by indigobirds. Like other nests of the firefinch (Hustler 1998), some were built in old weaver nests and others were built on the ground. (1) On 16 April 1996, two eggs appeared in one day in a ground nest. In the clutch of four, one egg was large and rounded,  $16.4 \times 12.4$  mm, the other small and pointed,  $15.9 \times 11.9$ . The large egg was the size of a *Vidua* egg, and the small ones the size of firefinch eggs (Hustler 1998, Payne *et al.* 2000); the clutch was taken by a predator. (2) In a nest built in an old weaver nest in February, a pink nestling hatched 3 days before the rest of the brood but later disappeared. (3) On 7 April, a nest built on the ground in a thicket had one pink-skinned nestling and three black-skinned nestlings. The black nestlings remained black until their feathers erupted; the pink nestling hatched at least 2 days before the rest of the brood (Fig. 1B). The brood was taken by a predator. (4) In February 1997, a nest was found in an old weaver nest in a reedbed away from riparian vegetation. It had a large nestling with pale pink skin, a yellow palate with three black spots, a gape with a pair of white papillae blue at the base, as in *V. chalybeata* and its firefinch host *L. senegala*. The nestling disappeared. (5) On 24 February an old nest of Holub's Golden Weaver *Ploceus xanthops* in a palm grove had an adult *L. nitidula* on a nest with 5 eggs; on 6 May it had a pale pink *Vidua* nestling. (6) On 11 March in a reed-bed, a fledged brood of *L. nitidula* had a fledgling indigobird with white gape papillae.

(7) On 24 February 1998, we flushed a brooding *L. nitidula* from its nest built in an old weaver nest. The firefinch nest had a large egg ( $15.3 \times 12.6$ ) and three small eggs ( $15.1 \times 11.9$ ,  $15.5 \times 11.3$ ,  $15.5 \times 11.3$ ); another large egg was laid later but did not hatch. The large egg hatched a pink *Vidua* nestling and the small eggs hatched black-skinned firefinch nestlings that died from day to day, as did the *Vidua* due to a tropical nest fly. (8) On 27 February, a firefinch nest in an old Village Weaver *Ploceus cucullatus* nest had a single pink *Vidua* nestling about 5 days of age; on next nest check it was gone. (9) On 29 March, a nest in an old weaver nest had four small firefinch eggs and a large *Vidua* egg. The nest was lost when the river flooded. (10) On 20 February 2000, a nest in an old weaver nest on the island had three small firefinch eggs and a large *Vidua* egg.

Of 94 nests of *L. nitidula* that were built in old weaver *Ploceus* spp. nests in reed-beds, palms and thickets examined through 1998, seven were parasitized with *Vidua* eggs or nestlings. Eggs were identified by size (mean,  $15.1 \times 11.4$  mm in 16 known *L. nitidula*, Hustler 1998;  $15.8 \times 12.6$  mm in 21 known *V. chalybeata*, Morel 1973, Payne 1977, Payne *et al.* 2000) or by their hatched young. Three nests had a pale, pink-skinned nestling with a mouth pattern like that of *V. chalybeata* and *L. senegala*, and not like that of *L. nitidula*. Nestling *V. chalybeata* and *L. senegala* have a yellow palate with three black spots, a pair of blue bordered white papillae at the gape (Morel 1973, Payne 1973, 1982), whereas nestling *L. nitidula* have a pinkish-white palate with three black spots and white papillae (Nicolai 1987, Hustler 1998). The skin and tarsi of nestling *L. senegala* are pink, whereas those of *L. nitidula* are black. Molecular genetics analysis confirmed that the three cases that we tested of suspected indigobird nestlings (large size, first hatched, pink skin, yellow palate) in nests of *L. nitidula* in fact were indigobirds; mtDNA sequences of these birds were compared with all other species of *Vidua* and all species of estrildid finches in southern Africa. In the same area we observed nests of the normal host *L. senegala* that were parasitized by nestling indigobirds of the same appearance. In all observations the nestling indigobirds in the nests of *L. nitidula* mimicked the mouth and skin of nestling *L. senegala* and not *L. nitidula* (Fig. 1).

### Microsatellites and kinship

Based on microsatellite analyses, many indigobirds that were associated with *L. nitidula* at Kazungula

appear to be close relatives. In one case, a male, a female and a nestling, all on the island, were identified as parents and offspring. The male was recorded mimicking *L. nitidula* song in April 1996 and again in February 1998. The female visited this male repeatedly over a 3-week period in 1998 and copulated with him. She also flew to call-sites of the other two mimics of *L. nitidula* on the island, but she was not seen at the call-site of the male that mimicked songs of *L. senegala*, even though that call-site was nearby (200 m). The nestling was found in a *L. nitidula* nest on the island about 2 weeks later. As expected for mother and offspring, the female and nestling shared the same mtDNA haplotype. Two other indigobird nestlings (sampled in 1997 and 1998) from *L. nitidula* nests on the island were identified as possible offspring of two other adult males mimicking *L. nitidula* song (sampled in 1996 and 1998, respectively). One of these males was also linked to two additional adult males (both with *L. nitidula* song), the genotypes and mtDNA haplotypes of which were consistent with their being older siblings of the sampled nestling. No potential mothers were identified in these cases, but little effort was made to capture females during our field study.

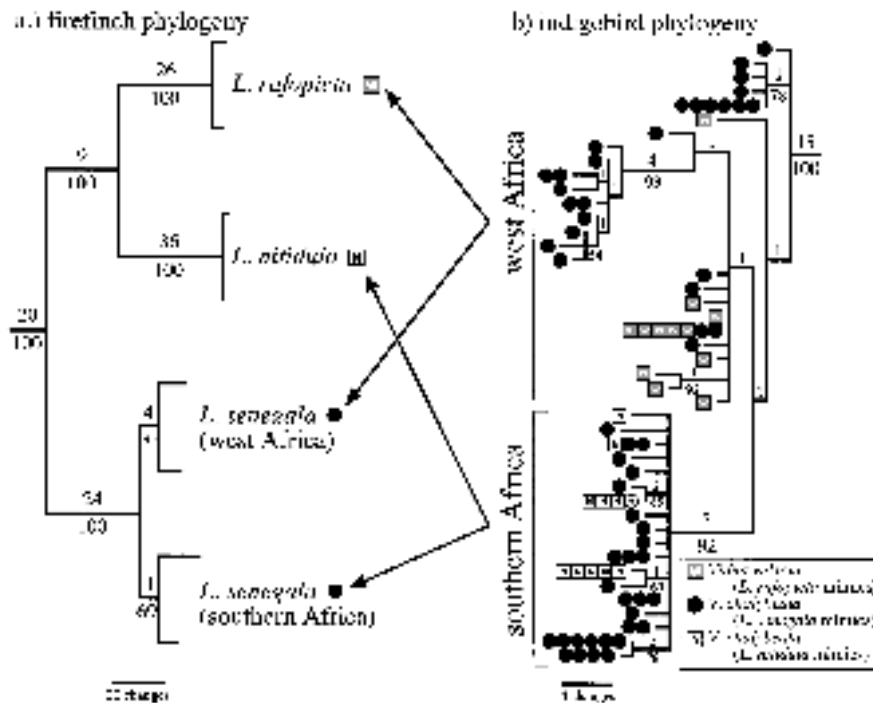
In general, individuals identified as possible parents and offspring were also identified as potential close relatives using the KINSHIP program ( $P < 0.001$ ). None of the indigobirds mimicking *L. senegala* at Kazungula was identified as parents or offspring of individuals associated with *L. nitidula*, or vice versa; and no such pairs were found to be probable close relatives.

With larger samples of birds, it may be possible to estimate the genetically effective population size, population structure and historical processes of divergence in indigobird populations using microsatellite techniques (e.g. Smith *et al.* 1997, Beaumont & Bruford 1999).

### Molecular genetics and species relationships

Phylogenetic analysis of mtDNA sequences indicate that the indigobirds that mimic and parasitize *L. nitidula* in the Zambezi region are more closely related to the southern *V. chalybeata* mimics of *L. senegala* than they are to the west African *V. wilsoni* mimics of *L. rufopicta* (Fig. 3). In addition, the genetic divergence between the indigobirds is much less than between the host firefinch species. Indigobirds





**Figure 3.** mtDNA gene tree for brood-parasitic indigobirds *Vidua chalybeata* and *V. wilsoni* (b) compared with the phylogeny of their firefinch *Lagonosticta* hosts (a). Outgroups used to root the trees (not shown) included Straw-tailed Whydah *Vidua fischeri* and Shaft-tailed Whydah *V. regia* for the indigobirds, and four additional firefinch *Lagonosticta* species and Brown Twinspot *Clytospiza monteiri* for the hosts. A strict consensus of 5616 trees of length 148 (CI = 0.74) based on 1103 aligned nucleotide positions is shown for the indigobirds. Both trees are drawn with branch lengths proportional to the number of changes, but note the different scales, reflecting the different time depth of phylogenies of host and parasite. The single most parsimonious host tree based on 1682 aligned nucleotide positions was 699 steps (CI = 0.68). In the indigobird tree, each symbol represents an individual bird, and birds on the same line had identical mitochondrial haplotypes. Decay indices and bootstrap values are shown above and below each node. One *V. chalybeata* from southern Africa (indicated by an asterisk) mimicked the songs of African Firefinch *L. rubricata*, as described earlier (Payne *et al.* 1993).

in southern and west Africa are separated by an average genetic distance (Kimura 1980, Steel *et al.* 1996) of only 1.4%, compared to 9.2% between *L. senegala* and the *L. nitidula* – *L. rufopicta* clade. Smaller genetic distances between indigobird species than in firefinches indicate a more recent divergence of the brood-parasites than of their corresponding host species. The observations support the colonization hypothesis, and they allow us to reject the cospeciation hypothesis. In an earlier molecular genetics analysis of indigobird species in southern and western Africa, the data indicated host switch and colonization by indigobirds from one host species to another host species. Although the direction of host switch was not clear, the morphologically distinct species within a region were more likely to share genetic sequences with each other than either did to its ecological counterpart in the other region (Klein *et al.* 1993, Klein & Payne 1998).

## DISCUSSION

Our observations on the upper Zambezi show the first known population-level switch of a *Vidua* brood parasite to a novel host species. The *V. chalybeata* that now parasitize *L. nitidula* are derived from a population that had parasitized *L. senegala*. The direction of this host switch was from *L. senegala* to *L. nitidula*, rather than in the other direction, as indicated by the following observations. Nestling indigobirds in *L. nitidula* nests had mouth markings similar to those of *L. senegala* rather than *L. nitidula*. Also, *V. chalybeata* is a widespread and morphologically distinct brood parasite of *L. senegala*, which is common in west, east, central and southern Africa. In contrast, *L. nitidula* has a limited distributional range, mainly outside that of the red-billed indigobirds *V. c. amauropteryx*. *L. nitidula* has been known from the upper Zambezi for more than 60 years and was seen near Kazungula at least 20 years ago,



whereas red-billed *V. c. amauropteryx* is known upstream on the upper Zambezi only as far as 50 km from Kazungula in western Zambia (Winterbottom 1942, 1956, Benson *et al.* 1971, Payne 1973, Irwin 1981). Based on our capture–mark–recaptures in 1997 we estimated about 100 pairs of *L. nitidula* on Kazungula island, which is free of the large terrestrial herbivores (elephant, buffalo) that destroy reed-bed habitat on the mainland.

This switch and the establishment of a new breeding population of indigobirds on the upper Zambezi have occurred without geographical isolation. Both firefinch species nest on the island, and indigobird song mimics of both firefinch species were observed and recorded on the island. Individual indigobirds are not restricted to the island, as we saw them fly 400–600 m across the river between island and mainland.

The age of this host switch is unknown. *V. chalybeata* may have parasitized *L. nitidula* for a long time, though not long enough to undergo significant genetic differentiation or to evolve mimetic mouth markings. Nicolai (1972) had two captive indigobirds that mimicked songs of *L. nitidula* and was informed that they originated from Angola and southern Zaire. Based on their song he described them as a new species ('*V. incognita*') but did not distinguish them from other indigobirds that have a white bill and red feet, such as *Vidua chalybeata* in Botswana and Angola (*V. c. okavangoensis*) or southeastern Zaire and Tanzania (*V. c. centralis*) (Payne 1973, 1982). The captives may have been from other populations of *V. chalybeata* that switched hosts and now mimic and parasitize *L. nitidula*; they differed from the upper Zambezi birds in their white bill (red in Zambezi birds). More recently, Vernon (1975) saw a female indigobird with a breeding pair of *L. nitidula*, as if searching for its nest, at Victoria Falls, not far from our observation sites.

In southern Africa, about 1% of male indigobirds (in a sample of 484 males) mimicked the song of an estrildid finch species other than the usual host, but in each local sample only one male had the odd song and the others in the local population had songs normal for their species (Payne 1973, Payne *et al.* 1993). This proportion of males with odd songs gives an estimate (1%) of the rate at which females lay in the nests of alternate host species and have nestlings that are reared successfully by the alternate foster species. If several females are successful when they parasitize a novel host in a local area, a new indigobird population may become established. This process has

been observed in a captive population. When indigobirds are reared under an experimental foster species, the males learn the songs of their experimental foster species rather than the songs of the normal firefinch host species that lived in the same aviary. In addition, the females imprint to the experimental foster species. When mature, the females parasitize the foster species that reared them, rather than the normal host species, and they are attracted to the songs of males that mimic their experimental foster species, rather than to the songs of males that mimic their normal host the firefinch (Payne *et al.* 1998, 2000). Our genetic data suggest that the indigobirds that parasitize *L. nitidula* and mimic their songs in the Zambezi region are not simply a few individuals reared by an atypical host. The four differing mitochondrial haplotypes indicate that at least four female indigobirds founded the new behavioural population. Also, the indigobirds that mimic songs of *L. nitidula* have been observed and recorded there over several years.

The success of the behavioural switch of *V. chalybeata* from *L. senegala* to *L. nitidula* may have been facilitated by similar features of the mouth pattern of nestlings of the two firefinch species. The pattern is the same and the mouth differs only in the palate colour, though the nestling skin colour differs considerably between *L. senegala* and *L. nitidula* (Fig. 1C,E).

The process that leads to speciation is often more rapid than the process of lineage sorting, in which the differential extinction of ancestral mtDNA lineages is completed in the descendant species (Avice 1994). All indigobird species, including *V. wilsoni*, have a relatively recent origin and have retained some of the ancestral genetic mtDNA polymorphisms and therefore are incompletely differentiated genetically (Fig. 2). Nevertheless, *V. wilsoni* differs from the indigobird population mimicking *L. nitidula* in being clearly distinct in morphology, in mimicking the mouth patterns of their current host species, and in showing significantly different frequencies of mtDNA haplotypes than other species in their own region.

Indigobirds provide a contrast to Cuckoos *Cuculus canorus*, a brood-parasitic species with no behaviour by which males signal the identity of their foster species, and where males and females mate randomly in respect to the host species (Marchetti *et al.* 1998, Gibbs *et al.* 2000). In the indigobirds, both males and females imprint on their foster parents, males mimic the songs of the foster species, and females are

selectively attracted to male song that mimics their own foster species, and the mimicry song forms the basis of mate recognition, as reasoned by Nicolai (1964) and tested in our experiments with captive-bred indigobirds *V. chalybeata*. Our finding that nestling indigobirds in *L. nitidula* nests were the offspring of male song mimics of *L. nitidula* is consistent with recent experimental studies demonstrating the importance of behavioural imprinting mechanisms in the indigobirds (Payne *et al.* 1998, 2000). Our result demonstrates for a natural population that a female indigobird mating with a male mimicking *L. nitidula* song also chooses *L. nitidula* nests in which to lay her eggs. Imprinting on the host species by both male and female indigobirds provides a mechanism for rapid reproductive isolation after the colonization of a new host species. Although the sample size of individuals was small, the results of the microsatellite analyses are consistent with the indigobirds at Kazungula being separated into two distinct non-interbreeding populations, one associated with *L. senegala* and the other with *L. nitidula*.

Imprinting to the same host species attracts the female to a male that sings the songs like those of her foster father, and mating that results from this process is likely to produce nestlings with mouth markings which allow parental care by their shared host species. In indigobirds this behaviour has led to genetic change, with selection for nestling mouth mimicry and with speciation of birds reared by different host species. Because geographical isolation is not necessary in this process, host switching may lead to sympatric speciation in the parasitic finches (Payne 1997).

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