

Online Supplementary Information – Sorenson, Sefc, and Payne (2003)

Tables 1-4. Pair-wise population differentiation values and p-values. P-values in bold remain significant at the 0.05 level using the method of Benjamini and Hochberg (1995) to control the rate at which null hypotheses are falsely rejected.

Table 1. Pair-wise \bar{D}_{ST} values for mtDNA differentiation between West African indigobird species. \bar{D}_{ST} values above the diagonal, p-values below. Overall $\bar{D}_{ST} = 0.41$ ($p < 0.0001$).

	<i>camerunensis</i>	<i>chalybeata</i>	<i>larvaticola</i>	<i>maryae</i>	<i>nigeriae</i>	<i>raricola</i>	<i>wilsoni</i>
<i>camerunensis</i>	—	0.63	0.23	0.16	0.45	0.39	0.22
<i>chalybeata</i>	<0.001	—	0.54	0.53	0.60	0.63	0.51
<i>larvaticola</i>	0.0024	<0.001	—	~0	0.19	0.09	~0
<i>maryae</i>	0.014	<0.001	n.s.	—	0.26	0.14	~0
<i>nigeriae</i>	<0.001	<0.001	<0.001	<0.001	—	0.26	0.21
<i>raricola</i>	<0.001	<0.001	0.026	0.012	<0.001	—	0.09
<i>wilsoni</i>	0.0016	<0.001	n.s.	n.s.	<0.001	0.012	—

Table 2. Pair-wise \bar{D}_{ST} values for mtDNA differentiation between southern indigobird species. \bar{D}_{ST} values above the diagonal, p-values below. Overall $\bar{D}_{ST} = 0.043$ ($p = 0.0014$).

	<i>chalybeata</i>	<i>funerea</i>	<i>codringtoni</i>	<i>purpurascens</i>
<i>chalybeata</i>	—	0.062	0.084	0.029
<i>funerea</i>	0.0095	—	0.054	~0
<i>codringtoni</i>	0.0062	0.036	—	~0
<i>purpurascens</i>	0.041	n.s.	n.s.	—

Table 3. Pair-wise R_{ST} values for nuclear microsatellite differentiation between West African indigobird species. R_{ST} values above the diagonal, p-values below. Overall $R_{ST} = 0.034$ ($p = 0.0015$).

	<i>camerunensis</i>	<i>chalybeata</i>	<i>larvaticola</i>	<i>maryae</i>	<i>nigeriae</i>	<i>raricola</i>	<i>wilsoni</i>
<i>camerunensis</i>	—	0.034	0.030	0.068	0.086	0.024	0.024
<i>chalybeata</i>	n.s.	—	0.024	0.054	0.036	~0	0.050
<i>larvaticola</i>	n.s.	n.s.	—	~0	0.023	0.014	0.023
<i>maryae</i>	0.044	0.076	n.s.	—	0.018	0.017	0.058
<i>nigeriae</i>	0.0018	0.059	n.s.	n.s.	—	0.040	0.075
<i>raricola</i>	0.088	n.s.	n.s.	n.s.	0.026	—	0.037
<i>wilsoni</i>	n.s.	0.060	n.s.	0.059	0.0064	0.064	—

Table 4. Pair-wise R_{ST} values for nuclear microsatellite differentiation between southern indigobird species. R_{ST} values above the diagonal, p-values below. Overall $R_{ST} = 0.027$ ($p < 0.001$).

	<i>chalybeata</i>	<i>funerea</i>	<i>codringtoni</i>	<i>purpurascens</i>
<i>chalybeata</i>	—	0.037	0.035	0.030
<i>funerea</i>	0.003	—	0.036	0.027
<i>codringtoni</i>	0.019	0.024	—	~0
<i>purpurascens</i>	0.009	0.023	n.s.	—

Figures 1-2, Table 5: Additional evidence suggesting a recent origin of extant indigobird species. In general, nuclear microsatellite loci and nuclear intron sequences are consistent with mtDNA in suggesting limited genetic variation and a lack of divergent genetic lineages within indigobirds. Divergent alleles or haplotypes would be expected for at least some loci if indigobirds had been in long and continuous association with their current host species.

Figure 1 (next page). Distribution of allele sizes at 11 microsatellite loci (Indigo 7 – Indigo 41) in West African (blue-top) and southern African (purple-bottom) indigobirds. Allele size in base pairs including flanking regions is on the x-axis. The frequency of each allele is proportional to the area of the corresponding circle. N = 117 West African indigobirds (234 alleles per locus), N = 98 southern indigobirds (196 alleles per locus).

Allele size distributions for many loci are unimodal or at most bimodal and the most common alleles tend to lie near the center of the size distribution. This pattern is similar to that observed for mtDNA haplotypes – i.e., a common ancestral allele (or haplotype) from which others are derived by a small number of mutations – and suggests a recent origin for these populations. In addition, southern and western birds exhibit a significant degree of differentiation that is consistent with the moderate divergence observed in their mtDNA. There has not been sufficient time, however, for mutation to generate a full range of allele sizes in each region (see Balloux et al. 2000).

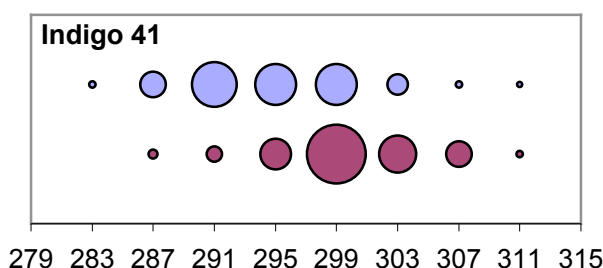
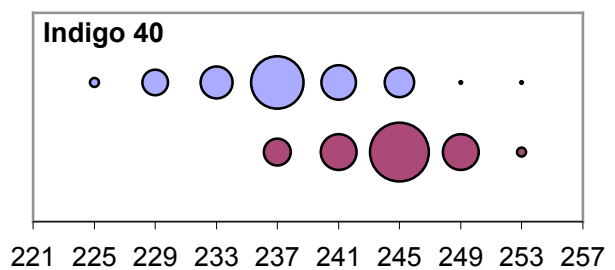
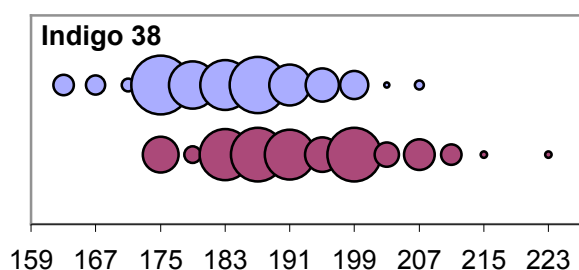
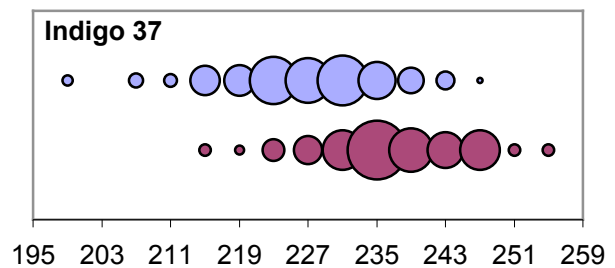
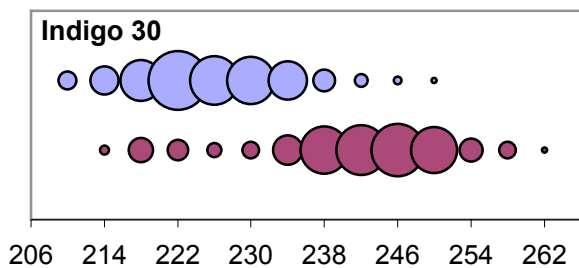
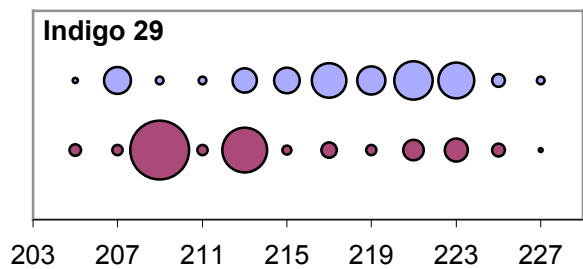
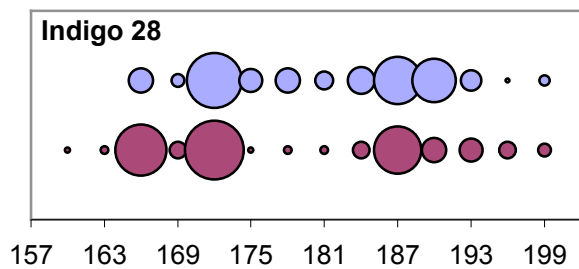
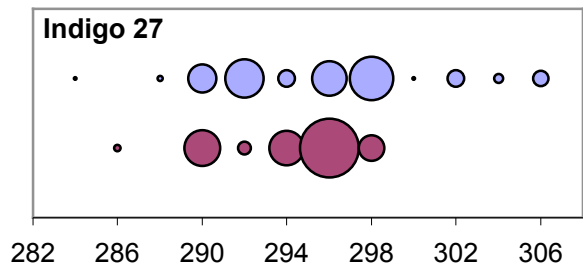
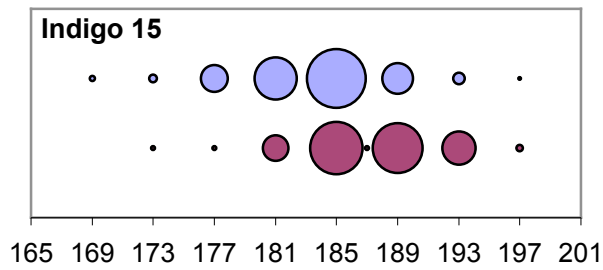
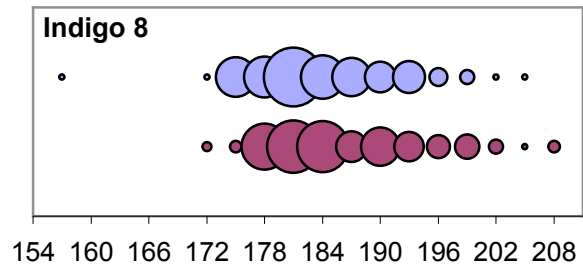
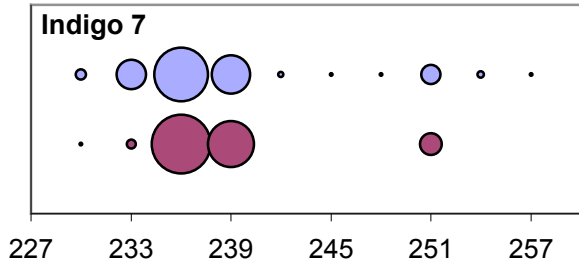
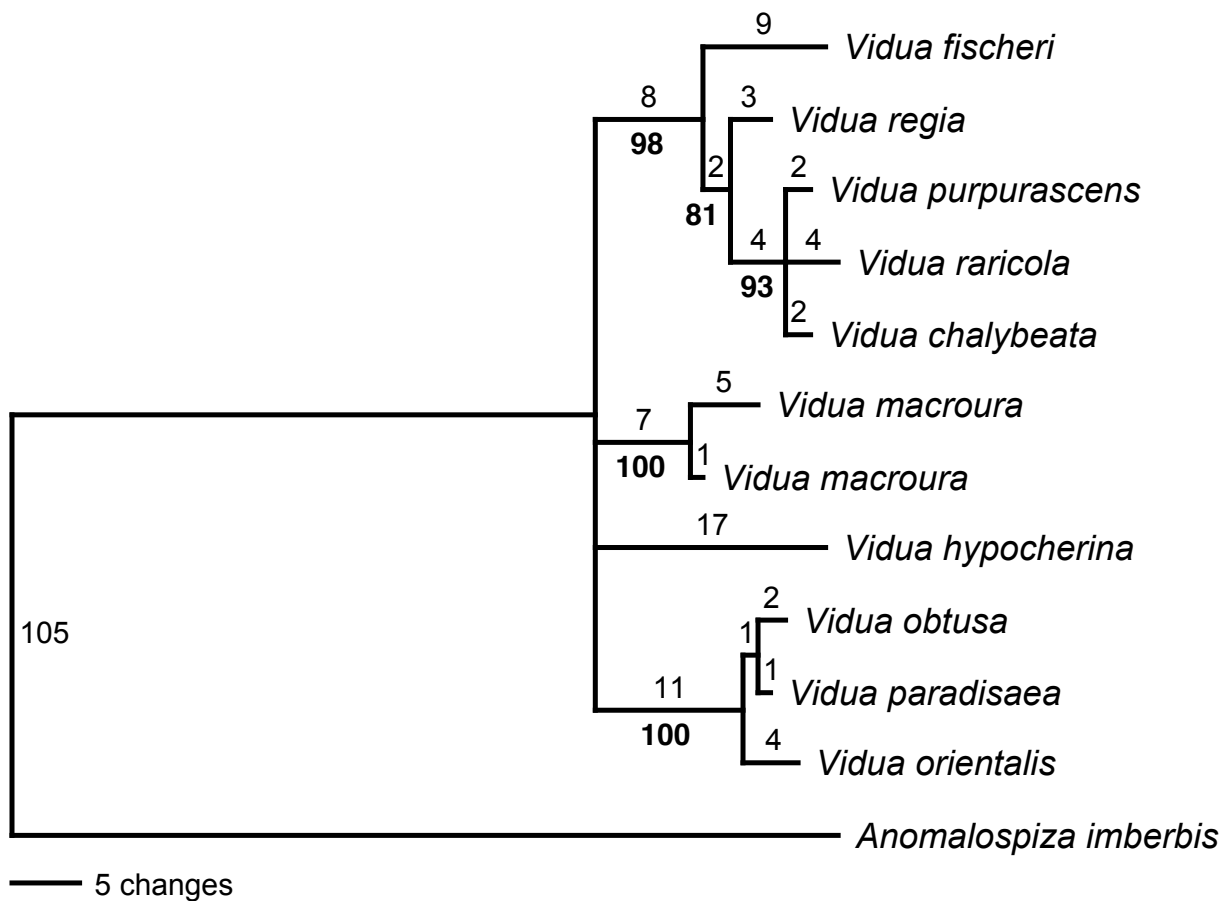


Figure 2. Phylogeny of parasitic finches based on 2908 aligned nucleotide positions from a combined analysis of 6 introns from 5 independent nuclear genes (introns 3 and 9 of phosphoenolpyruvate carboxykinase; intron 5 of transforming growth factor beta 2; intron 5 of laminin receptor precursor / p40; intron 11 of glyceraldehyde-3-phosphate dehydrogenase; intron 8 of alpha-enolase) (Sorenson, Balakrishnan and Payne, unpublished data). Gaps were treated as a fifth character state but with multiple base deletions treated as single characters. The tree shown is a strict consensus of nine equally parsimonious trees of length 248 (consistency index = 0.95), with branch lengths and bootstrap values above and below each node, respectively. The tree is generally congruent with the mtDNA phylogeny and indicates a close relationship and limited genetic divergence among three indigobirds, including one from southern Africa (*V. purpurascens*) and two from West Africa (*V. raricola*, *V. chalybeata*).



— 5 changes

Table 5. Comparison of microsatellite heterozygosity, allelic diversity, and differentiation values (R_{st}) for all western and all southern indigobirds, respectively. Heterozygosity and allelic diversity are lower in southern birds on average and in 7 of 11 individual loci. This is consistent with a lower level of mtDNA haplotype diversity in southern birds (see Figure 2 in main paper) and a more recent origin of southern species. N = 117 West African indigobirds (234 alleles per locus), N = 98 southern indigobirds (196 alleles per locus). No individual locus shows high levels of population differentiation, consistent with a recent origin for all indigobird species.

Locus	heterozygosity		allelic diversity		R_{st}	
	west	south	west	South	west	south
Ind7	0.69	0.58	1.45	1.17	0.008	0.027
Ind8	0.85	0.86	2.36	2.61	~0	0.029
Ind15	0.70	0.71	1.06	1.01	0.008	~0
Ind27	0.81	0.68	2.27	1.34	0.0005	0.013
Ind28	0.84	0.81	2.91	3.80	~0	0.026
Ind29	0.87	0.74	2.81	2.65	0.059	0.006
Ind30	0.84	0.86	1.94	2.56	0.054	0.076
Ind37	0.86	0.84	2.27	2.05	0.072	0.071
Ind38	0.86	0.87	2.28	2.35	0.081	0.076
Ind40	0.75	0.68	1.30	0.80	0.015	~0
Ind41	0.77	0.70	1.36	1.07	0.102	0.007
average	0.80	0.76	2.00	1.95	0.034	0.027

Table 6. Parasitic *Vidua* and their respective hosts. Principal hosts are indicated by an asterisk. Other host species are either parasitized in a small part of the parasitic species range or are infrequent hosts with relatively few records of parasitism (Payne 1996, 1997, 1998, in press; Payne and Payne, 2002). Morphologically indistinguishable indigobirds associated with different hosts are treated as single species.

Parasite species	Host species	Host species	Host species
<i>Vidua chalybeata</i>	Village Indigobird	* <i>Lagonosticta senegala</i>	Red-billed Firefinch
		<i>L. nitidula</i>	Brown Firefinch
<i>V. codringtoni</i>	Peters' Twinspot Indigobird	* <i>Hypargos niveoguttatus</i>	Peters' Twinspot
		<i>H. margaritatus</i>	Rosy Twinspot
<i>V. funerea</i>	Dusky Indigobird	* <i>L. rubricata</i>	African Firefinch
<i>V. purpurascens</i>	Purple Indigobird	* <i>L. rhodopareia</i>	Pink-backed Firefinch
<i>V. larvaticola</i>	Black-faced Firefinch Indigobird	* <i>L. larvata</i>	Black-faced Firefinch
		<i>L. virata</i>	Mali Firefinch
<i>V. maryae</i>	Jos Plateau Indigobird	* <i>L. sanguinodorsalis</i>	Rock Firefinch
<i>V. wilsoni</i>	Bar-breasted Firefinch Indigobird	* <i>L. rufopicta</i>	Bar-breasted Firefinch
<i>V. camerunensis</i>	Fonio Indigobird	* <i>L. rara</i>	Black-bellied Firefinch
		<i>L. rubricata</i>	Blue-billed Firefinch
		<i>Clytospiza monteiri</i>	Brown Twinspot
		<i>Euschistospiza dybowski</i>	Dybowski's Twinspot
<i>V. nigeriae</i>	Quail-finch Indigobird	* <i>Ortygospiza atricollis</i>	Quail-finch
<i>V. raricola</i>	Goldbreast Indigobird	* <i>Amandava subflava</i>	Goldbreast
<i>V. regia</i>	Shaft-tailed Whydah	* <i>Granatina granatina</i>	Violet-eared Waxbill
<i>V. fischeri</i>	Straw-tailed Whydah	* <i>G. ianthinogaster</i>	Purple Grenadier
<i>V. hypocherina</i>	Steel-blue Whydah	* <i>Estrilda erythronotos</i>	Black-faced Waxbill
		* <i>E. charmosyna</i>	Pink Black-faced Waxbill
<i>V. macroura</i>	Pin-tailed Whydah	* <i>Estrilda astrild</i>	Common Waxbill
		* <i>E. melpoda</i>	Orange-cheeked Waxbill
		* <i>E. troglodytes</i>	Black-rumped Waxbill
		* <i>E. rhodopyga</i>	Red-rumped Waxbill
		* <i>E. paludicola</i>	Fawn-breasted Waxbill
		* <i>Coccopygia melanotis</i>	Swee Waxbill
		* <i>C. quartinia</i>	East African Swee
<i>V. paradisaea</i>	Paradise Whydah	* <i>Pytilia melba</i>	Melba Finch
<i>V. obtusa</i>	Broad-tailed Paradise Whydah	* <i>P. afra</i>	Orange-winged Pytilia
<i>V. orientalis</i>	Sahel Paradise Whydah	* <i>P. melba citerior</i>	Melba Finch
<i>V. interjecta</i>	Exclamatory Paradise Whydah	* <i>P. phoenicoptera</i>	Red-winged Pytilia
		<i>P. hypogrammica</i>	Yellow-winged Pytilia
		<i>P. lineata</i>	Red-faced Pytilia
<i>V. togoensis</i>	Togo Paradise Whydah	* <i>P. hypogrammica</i>	Yellow-winged Pytilia

Species concepts and criteria:

We apply to indigobirds the general lineage concept of species (de Queiroz 1998) which recognizes as species segments of independent evolutionary lineages. In sexual organisms, species-level lineages are unified by reproduction and share a common evolutionary trajectory that is independent from other species-level lineages. This concept does not require complete reproductive isolation, but only a degree of isolation sufficient to allow independent evolution. Genetic, behavioral, and morphological data suggest that indigobird species have independent evolutionary trajectories – as evidenced perhaps most importantly by the evolution of mouth markings that mimic different host species – and that they are largely, but not completely, reproductively isolated.

Also following de Queiroz (1998), we distinguish between species concepts and the operational criteria used to recognize species. For indigobirds, we recognize species on the basis of discontinuous variation in juvenile mouth markings and/or adult morphology as well as strong behavioral evidence for reproductive isolation and assortative mating. In adult males, differences among species include plumage color, color of the primaries, bill color, color of the feet and legs, and body size. In some cases, females also can be distinguished based on morphology – e.g., *Vidua chalybeata* and *V. purpurascens* in southern Africa. Field observations of these two species suggest 100% assortative mating (Payne 1973). Likewise, the nearly perfect correspondence between male morphology and mimicry song (>99%) provides additional evidence for the integrity of these species, particularly in light of the recent captive experiments by Payne et al. (2000, 2002) that demonstrate host imprinting in both male and female indigobirds.

In cases where indigobirds associated with different hosts within the same region are not morphologically differentiated, we have continued to treat these cases as single species, pending additional evidence of morphological or genetic differentiation.

Indigobirds present a challenge to any narrow concept of species and some may consider the lack of reciprocal monophyly in mtDNA haplotypes and minimal differentiation in microsatellite loci a problem for the recognition of indigobirds as distinct species. In our view, a particular level of genetic differentiation at neutral genetic loci is not a necessary criterion for the recognition of species. Indeed, the expectation for recent sympatric speciation is minimal differentiation at neutral genetic markers. As Via (2001) notes: “Sympatrically derived species are expected to show profound genetic similarity, differing only at the handful of loci that are under disruptive/divergent selection or are associated with mate choice. This is a population genetic signature of sympatric speciation, distinguishing it from speciation in allopatry where genetic differences are expected to accumulate throughout the genome.” Even if hybridization among sympatric indigobirds is sufficiently high to impede the accumulation of significant genetic structure at neutral loci, this would not necessarily preclude their recognition as species so long as diversifying selection keeps their populations on independent evolutionary trajectories.

All indigobirds could be viewed as a single species only by adopting a narrow interpretation of the biological species concept – one allowing no hybridization or genetic introgression

between species. This approach, however, would contribute little to describing the essential aspects of the indigobird system. Indigobird are species, but with admittedly fuzzy boundaries.

The morphology of indigobird species and their host associations have been described in previous publications (Payne 1973, 1982, 1996, 1998; Payne and Payne 1994, 1995; Payne et al. 1992, 2002). The current list of indigobird species and known hosts might very well change with additional research but we think the current taxonomy provides the best possible summary of what is known about the morphological diversity of indigobirds.

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