

**Limits to host colonisation and speciation in a radiation of parasitic finches**

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56 **Data accessibility statement:**

57  
58 Raw data and associated R scripts will be uploaded to Dryad on acceptance of the  
59 manuscript  
60

# 1 Limits to host colonisation and speciation in a radiation of parasitic

## 2 finches

### 10 Abstract

13 Parasite lineages vary widely in species richness. In some clades, speciation is  
14 linked to the colonisation of new hosts. This is the case in the indigobirds and  
15 whydahs (*Vidua*), brood-parasitic finches whose nestlings mimic the phenotypes of  
16 their specific hosts. To understand the factors limiting host colonisation, and  
17 therefore speciation, we simulated the colonisation of a host using cross-fostering  
18 experiments in the field. Despite DNA barcoding suggesting that host species feed  
19 their chicks similar diets, nestling *Vidua* had low survival in their new host  
20 environment. Nestling *Vidua* did not alter their begging calls plastically to match  
21 those of the new hosts, and were fed less compared to both host chicks and to *Vidua*  
22 chicks in their natural host nests. This suggests that a key hurdle in colonising new  
23 hosts is obtaining the right amount rather than the right type of food from host  
24 parents. This highlights the importance of mimetic nestling phenotypes in soliciting  
25 feeding from foster parents and may explain why successful colonisations tend to be  
26 of hosts closely-related to the ancestral one. That non-mimetic chicks are fed less  
27 but not actively rejected by host parents suggests how selection from hosts can be  
28 sufficiently intense to cause parasite adaptation, yet sufficiently relaxed that parasitic  
29 chicks can sometimes survive in and colonise new host environments even if they  
30 lack accurate mimetic phenotypes. The difficulties of soliciting sufficient food from  
31 novel foster parents, together with habitat filters, likely limit the colonisation of new  
32 hosts, and therefore speciation, in this parasite radiation.

**Lay summary (75 words)**

23 Why are some groups species-rich, while others are species-poor? For Africa's  
24 brood-parasitic finches, species richness is intimately tied to the colonisation of new  
25 host species. Using field experiments, we show that a major obstacle to host-  
26 switching, and therefore speciating, is obtaining the right *amount* of food from the  
27 new host parent rather than the right *type* of food. This is possible when parasite and  
28 host chicks have similar nestling begging displays.

For Review Only

## Introduction

29 Why are some adaptive radiations species-rich, while others species-poor (Schluter,  
30 2000)? To explain the extent of an adaptive radiation, we need to understand both  
31 what limits the colonisation of new niches, and how niche colonisation generates  
32 reproductive isolation between lineages (Stroud and Losos, 2016). Specialised  
33 parasite-host systems provide excellent opportunities to understand the forces  
34 limiting diversification, because hosts constitute discrete niches to which parasites  
35 must adapt. If parasite lineages speciate each time they colonise a new host, the  
36 species richness of the parasite clade will be directly related to the number of host  
37 species it has successfully colonised (Poulin and Morand, 2000). Therefore, to  
38 understand the species richness of such a parasite radiation, we must understand  
39 what has limited the colonisation of new hosts.

41 Close associations between speciation and host-switching have been found in  
42 several groups, including phytophagous butterflies (Fordyce, 2010; Hardy and Otto,  
43 2014; Janz et al., 2006), *Rhagoletis* flies (Bush, 1969; Filchak et al., 2000; McPheron  
44 et al., 1988) and fish ectoparasites (Ziętara and Lumme, 2002). Among vertebrates,  
45 one of the most compelling examples of speciation via host-switching comes from  
46 the indigobirds and whydahs (genus *Vidua*) (Sorenson et al., 2003). The genus  
47 *Vidua* is a radiation of 19 brood-parasitic finch species (Payne, 2010b). *Vidua* are  
48 host specialists, each laying their eggs predominantly in the nest of a single host  
49 species of the grassfinch family (Estrildidae) (Payne, 2010a).

51 Speciation is linked to host colonisation in *Vidua* finches because of their remarkable  
52 capacity to imprint on hosts (Payne et al., 1998; Payne et al., 2000; Sorenson et al.,

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2  
3 53 2003). Males of most *Vidua* species incorporate elements of their host species'  
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5 54 vocalisations into their own displays as adults (Payne et al., 1998). Female *Vidua* are  
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7 55 attracted to males who sing like their natal host, and prefer to lay eggs in a nest of  
8  
9 56 that same host species (Payne et al., 2000). Therefore, male display, female mating  
10  
11 57 preference and female host preference are all strongly influenced by the host  
12  
13 58 environment in which the bird developed. The result is that male and female *Vidua*  
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15 59 raised by the same host tend to breed with one another, and parasite-host  
16  
17 60 associations are maintained across generations (Payne et al., 2000). This imprinting  
18  
19 61 mechanism means that if a female lays her egg in the nest of a previously  
20  
21 62 unparasitised host species, and her offspring survive in the new host environment,  
22  
23 63 they have the potential to initiate a new, reproductively isolated, lineage of *Vidua*  
24  
25 64 (Sorenson et al., 2003). Patterns of host colonisation are non-random and *Vidua*  
26  
27 65 generally colonise hosts in the same clade (and usually the same genus) as their  
28  
29 66 ancestral host, a pattern termed "clade-limited colonisation" (Sorenson et al., 2004).  
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37 67  
38 68 The nestlings of many *Vidua* species possess specialist mimetic adaptations to their  
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40 69 hosts (Jamie et al., 2020; Payne, 2005). The host family, the Estrildidae, is unusual  
41  
42 70 among birds in having nestling phenotypes that vary extensively among species  
43  
44 71 (Payne, 2005). The young of each species have a characteristic combination of  
45  
46 72 mouth markings, skin colour, begging calls, head movements and natal down (Jamie  
47  
48 73 et al., 2020; Payne, 2005). The nestlings of several *Vidua* species mimic the specific  
49  
50 74 appearance, vocalisations and movements of their host young (Jamie et al., 2020;  
51  
52 75 Neunzig, 1929; Nicolai, 1964, 1974, 1989, 1991; Schuetz, 2005b, Payne, 2005;  
53  
54 76 Payne and Payne, 2002). This is a form of "aggressive signal mimicry" in which  
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56 77 signals of the model (host chicks) are mimicked by the parasitic chick, who  
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3 78 deceptively advertises a reward to receivers (host parents) (Jamie, 2017b). Previous  
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5 79 work, in which the appearance of host nestlings was experimentally manipulated  
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7 80 (Schuetz, 2005a) and host nestlings cross-fostered in aviaries (Payne et al., 2001),  
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9 81 suggests that these mouth markings are important for soliciting food from parents.  
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14 83 However, a key piece of the puzzle is missing. How can *Vidua* colonise new host  
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16 84 species when they initially lack the requisite mimetic adaptations to exploit them?  
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18 85 Furthermore, why do some, seemingly suitable, estrildid finch species remain  
19  
20 86 unparasitised? Answering these questions is key to explaining why there are only 19  
21  
22 87 species of *Vidua*, and not many more or fewer. In this paper, we investigate the  
23  
24 88 mechanisms that prevent *Vidua* from colonising apparently suitable hosts: 1) Do host  
25  
26 89 parents reject foreign eggs? 2) Do host parents discriminate against foreign chicks  
27  
28 90 and, if so, do they do so by actively removing them from the nest, or by feeding them  
29  
30 91 less food? 3) Do different potential host species feed their young different foods,  
31  
32 92 thus providing a barrier to colonisation? 4) Do parasite nestlings fail to match their  
33  
34 93 begging calls to those of their novel host?  
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42 94

43 95 To answer these questions, we simulated a colonisation attempt on a novel host by  
44  
45 96 experimentally transferring eggs of a *Vidua* species, the pin-tailed whydah (*V.*  
46  
47 97 *macroura*) from nests of its natural host, common waxbill (*Estrilda astrild*), to nests of  
48  
49 98 a related non-host species, blue waxbill (*Uraeginthus angolensis*) (Figure 1). As  
50  
51 99 control treatments, we also transferred eggs of the common waxbill into additional  
52  
53 100 nests of blue waxbills, and swapped blue waxbills between blue waxbill nests.  
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55 101 Additionally, we compared survival of whydahs in blue waxbill nests to their survival  
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3 102 in naturally parasitised common waxbill nests to explore the costs of shifting to a new  
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5 103 host.

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10 105 At our field site, pin-tailed whydahs are specialist hosts on common waxbills and only  
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12 106 rarely lay their eggs in the nests of other species (Payne, 2010b). Blue waxbills are  
13  
14 107 an ideal model of a novel host as they are a common estrildid species but are almost  
15  
16 108 never used as hosts by *Vidua*. The pin-tailed whydah's range overlaps widely with  
17  
18 109 that of the blue waxbill, and there is evidence that female pin-tailed whydahs do very  
19  
20 110 occasionally lay in the nests of blue waxbills (Hockey et al., 2005; Tarboton, 2011,  
21  
22 111 GAJ pers obs). While pin-tailed whydahs are unusual in that they are one of just two  
23  
24 112 species in the *Vidua* radiation not known to imitate the song of their host species  
25  
26 113 (Payne, 2010b), they possess the host-specific nestling adaptations found in many  
27  
28 114 other *Vidua* species, mimicking the mouth markings, begging calls and posture of  
29  
30 115 their natural host, common waxbill (Jamie et al., 2020). This means that the  
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32 116 mechanisms found to limit host colonisation at the nestling stage in this species likely  
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34 117 also operate in other members of the *Vidua* radiation.  
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42 119 To address questions (1) and (2), we monitored the survival, feeding, diet and  
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44 120 begging calls of experimentally transferred offspring. If nestling pin-tailed whydahs  
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46 121 possess additional adaptations to survive in foreign nest environments, such as  
47  
48 122 plasticity in key traits or tolerance of a greater diversity of nestling diets, then pin-  
49  
50 123 tailed whydahs should survive better than common waxbills in blue waxbill nests,  
51  
52 124 and survive as well as blue waxbill young. For question (3), we quantified diet using  
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54 125 DNA barcoding of crop samples and compared the development of nestling begging  
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56 126 calls in different host environments to test for plasticity. For question (4), we tested  
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3 127 whether pin-tailed whydah nestlings flexibly modify their calls so that they sound like  
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5 128 more blue waxbill nestlings when raised in a blue waxbill nest.  
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## 11 **Methods**

### 15 130 ***Transfer experiments simulating host colonisation***

17  
18 131 During January–April 2014–2017, we carried out transfer experiments within an area  
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20 132 of about 40 km<sup>2</sup> on and around Musumanene and Semahwa Farms (centred on  
21  
22 133 16°47'S, 26°54'E) in the Choma District of Zambia. The habitat is a mixture of  
23  
24 134 miombo woodland, agricultural fields and seasonally-flooded grasslands.  
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30 136 The experiment had three treatments: (i) pin-tailed whydah eggs transferred from  
31  
32 137 common waxbill to blue waxbill nests, (ii) blue waxbill eggs transferred to blue waxbill  
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34 138 nests, and (iii) common waxbill eggs transferred to blue waxbill nests. Additionally,  
35  
36 139 we monitored survival and feeding of pin-tailed whydah nestlings in naturally-  
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38 140 parasitised common waxbill nests. To minimise predation, eggs were taken from  
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40 141 their natural nest and incubated in a Brinsea Octagon 20 Advance EX Incubator at  
41  
42 142 36.7°C and 60% humidity. A day before they were due to hatch, eggs were fostered  
43  
44 143 to a blue waxbill nest. Occasionally (16/94 transfers), the offspring had to be fostered  
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46 144 as a chick freshly hatched in the incubator, rather than as an egg but this was not  
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48 145 found to influence the offspring's subsequent survival in the novel nest environment  
49  
50 146 (see Results). The modal clutch size of both common waxbill and blue waxbill nests  
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52 147 was five. No host egg was removed when an egg/hatchling was added, because pin-  
53  
54 148 tailed whydah females do not remove a host egg when they naturally parasitise a  
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56 149 nest (Tarboton, 2011). Experimental nests were visited every two or three days, and

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3 150 the number of eggs and chicks in the nest was recorded. For chicks, mass and  
4  
5 151 tarsus length were measured and the amount of food in the crop scored (methods  
6  
7 152 below). Mass was measured on digital scales to an accuracy of 0.1 or 0.01 g  
8  
9  
10 153 depending on the model of scale. Tarsus length was measured using dial callipers to  
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12 154 the nearest 0.1 mm.  
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17 156 All three species used in the study are common and widespread and experience  
18  
19 157 high levels of natural nest predation such that the experiments carried out for this  
20  
21 158 study will have negligible effects on their populations. Data were collected under the  
22  
23 159 research approval of the Department of National Parks and Wildlife in Zambia  
24  
25 160 (DNPW/8/27/1). Our sample sizes were chosen to allow the between-species effects  
26  
27 161 to be detected with a high degree of confidence while not including an unnecessarily  
28  
29 162 high number of individuals. As such it meets the ABS/ASAB guidelines and adheres  
30  
31 163 to the three R's of replacement, reduction and refinement (Buchanan et al., 2012).  
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36 164 ***Comparing survival of different species transferred to blue waxbill nests***  
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40 165 Survival analyses were carried out in the R statistical environment (R Development  
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42 166 Core Team, 2017) using the packages Survival (Therneau, 2015) and KMSurv  
43  
44 167 (Moeschberger and Yan, 2012). We monitored chick survival from the day the chick  
45  
46 168 hatched in the new host nest. Chick survival was judged to end at the midpoint  
47  
48 169 between the last day the chick was known to be alive and the first day the chick was  
49  
50 170 known to be absent. If the nest was still active, but the transferred chick was absent,  
51  
52 171 then the chick was assumed to have died. If the nest was abandoned at the point the  
53  
54 172 transferred chick was absent, then the data were right-censored. Right-censoring is  
55  
56 173 used when the event of interest has not occurred by the last observation (Mills,  
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3 174 2011). A Cox proportional hazards model was fitted to the survival data (Cox, 1972).  
4  
5 175 The co-variates in the initial model were: (i) transferred chick species, (ii) presence of  
6  
7 176 host nestmates, and (iii) foreign chick transferred as egg or as chick. The number of  
8  
9 177 nestmates over the course of a given transfer experiment ranged from 0 to 5 (mean  
10  
11 178 = 1.4). In most transfers (49 of 94), the transferred chick had no nestmates in the  
12  
13 179 foster nest. We therefore modelled nestmate presence as presence/absence.  
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20 181 ***Comparing the amount of food host parents fed transferred chicks of different***  
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22 182 ***species***  
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26 183 Crop size of the transferred chick was recorded at each nest visit. Nestling estrildid  
27  
28 184 crops are transparent, allowing easy visual inspection. Crops were scored as 0  
29  
30 185 (empty), 1 (trace amounts, < c. 20 seeds, no bulge in crop), 2 (> c. 20 seeds, slight  
31  
32 186 bulge) or 3 (> c. 50 seeds, large bulge). To assess whether crop sizes of chicks  
33  
34 187 differed depending on the species of chick transferred, two approaches were used:  
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37 188  
38  
39 189 First, the median crop size of the transferred chick over the first 7 days of survival in  
40  
41 190 the host nest was used as the response variable; c. 80% of common waxbill and pin-  
42  
43 191 tailed whydah chick mortality occurred in this period (Figure 2). A Kruskal-Wallis test  
44  
45 192 was used to test whether median crop size differed between the three species.  
46  
47 193 Median crop sizes were compared between species using a Dunn post-hoc test,  
48  
49 194 using the `dunn.test` function from the R package `dunn.test` and with Bonferroni  
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51 195 correction for multiple testing (Dinno, 2017).  
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3 197 Second, ordinal mixed-effect models were implemented in the R package Ordinal  
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5 198 (Christensen, 2015) using crop score as an ordinal response variable. In the full  
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7 199 model, the fixed effects were chick species and the number of nestmates.  
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9  
10 200 Transferred chick individual was a random effect nested within the nest of origin of  
11  
12 201 that transferred chick. We carried out stepwise elimination of non-significant co-  
13  
14 202 variates until only significant co-variates remained. The model was initially run to  
15  
16 203 include crop scores over the first 7 days of development, then re-run using crop  
17  
18 204 scores over the first 4, 5, 6 and 8 days of development to test whether the findings  
19  
20 205 from the first 7 days of development were robust.  
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25  
26 207 Ordinal mixed-effect models were used to compare crop size in pin-tailed whydahs  
27  
28 208 and common waxbills occurring in their natural nests (common waxbill nests) with  
29  
30 209 those experimentally transferred to blue waxbill nests. Data for pin-tailed whydahs  
31  
32 210 and common waxbills in common waxbill nests are observational, unlike the  
33  
34 211 experimental data from pin-tailed whydahs in blue waxbill nests. This was because  
35  
36 212 high levels of nest predation meant that all common waxbill nests found at the egg  
37  
38 213 stage were required as a source of eggs for transfer to blue waxbill nests. Therefore,  
39  
40 214 our data on pin-tailed whydahs in common waxbill nests do not account for the  
41  
42 215 effects of transferring an egg from one nest to another. However, when blue waxbill  
43  
44 216 eggs were transferred from their own nest to another blue waxbill nest, they still  
45  
46 217 showed high survival (about 76% of all chicks transferred survived to fledging),  
47  
48 218 suggesting that any effects of transferring eggs between nests are insufficient to  
49  
50 219 account for the differences observed in chick survival between common and blue  
51  
52 220 waxbill nests.  
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3 222 **Measuring nestling diet composition**  
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6 223 **Obtaining crop samples in field**  
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9 224 Nestling crops were sampled using the tube insertion method (Zann and Straw,  
10  
11 225 1983). The tube was inserted in the throat of the nestling and seeds were pushed  
12  
13 226 from the translucent crop into the tube. The contents were stored in 70% ethanol.  
14  
15 227 The process was repeated until about 20–30 seeds had been extracted. Chicks were  
16  
17 228 sampled around the time when the primaries first erupt from pin (approximately 6–7  
18  
19 229 days of age).  
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24  
25 231 We sampled crops of common waxbill, blue waxbill as well crops of six other  
26  
27 232 sympatric estrildid finch species: orange-winged pytilia (*Pytilia afra*), melba finch  
28  
29 233 (*Pytilia melba*), Jameson's firefinch (*Lagonosticta rhodopareia*), red-billed firefinch (*L.*  
30  
31 234 *senegala*), African quailfinch (*Ortygospiza atricollis*) and bronze mannikin  
32  
33 235 (*Spermestes cucullatus*). We sampled these other estrildid finch species in addition  
34  
35 236 to the two used in the experiments to assess variation in host diet across a broader  
36  
37 237 phylogenetic scale and to explore whether estrildids that hosts to *Vidua* have a  
38  
39 238 different diet from those that are not.  
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45  
46 240 **DNA barcoding of nestling crop contents**  
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49 241 Nestling estrildid finch crops contained almost exclusively plant seeds. DNA  
50  
51 242 barcoding of samples was carried out by Jonah Ventures (Boulder, Colorado;  
52  
53 243 jonahventures.com). The chloroplast trnL intron was amplified from DNA in each  
54  
55 244 sample using the c and h trnL primers (Taberlet et al., 2007). The total expected  
56  
57 245 amplicon length was 332bp (Jonah Ventures *in litt.*). A detailed protocol is described  
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1  
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3 246 in supplementary methods. We consulted with an expert botanist based in Zambia,  
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5 247 Mike Bingham, to validate the taxonomic identities assigned by DNA barcoding.  
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### 9 10 249 **Quantifying crop contents**

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13 250 DNA barcoding data resolution allowed analysis at the subfamily level and not the  
14  
15  
16 251 genus level, so each OTU was assigned to one of the four subfamilies identified (see  
17  
18 252 Results). For each sample, reads from OTUs mapping to the same sub-family were  
19  
20 253 summed together to give a measure of the total number of reads from each  
21  
22 254 subfamily, and expressed as a proportion of total reads (Craine et al., 2015;  
23  
24  
25 255 Willerslev et al., 2014). To test whether different estrildid species fed chicks different  
26  
27 256 proportions of seeds from each of the four families, non-metric dimensional scaling  
28  
29 257 (NMDS) was performed using the R package vegan (Oksanen et al., 2017).  
30  
31 258 Comparisons of diet between species were made using the function adonis, from the  
32  
33 259 R package vegan.  
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36 260

### 37 38 39 40 261 ***Begging call plasticity***

### 41 42 43 262 **Recording nestling begging calls**

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46 263 Chicks were placed in an artificial nest, and given several minutes for acclimation. To  
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48 264 stimulate begging, the chick was tapped gently with forceps on the bill. Recordings  
49  
50 265 were made using an Audio-Technica ATR35S tie-clip microphone or a Sennheiser  
51  
52 266 ME-66 shotgun microphone held approximately three cm away from the chick's  
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54  
55 267 mouth. Recordings were made for around two minutes or until at least ten seconds  
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57 268 of continuous begging were recorded.  
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60 269

## 270 **Analysing the effect of host environment on nestling begging calls**

271 We compared begging calls of nestling pin-tailed whydahs in natural common waxbill  
272 nests, to those transferred to blue waxbill nests. We identified four distinct call types  
273 both by listening to recordings and through visual inspection of sonograms (see  
274 Results). All four call types were detected in both pin-tailed whydahs developing in  
275 common waxbill nests, and pin-tailed whydahs transferred to blue waxbill nests. To  
276 analyse whether host environment influenced the stage in the nestling period at  
277 which each call type was made, we examined the stage in development at which  
278 chicks made each call type and compared this between pin-tailed whydahs raised in  
279 common and blue waxbill nests. We used chick tarsus length as a proxy for  
280 developmental stage, because for pin-tailed whydahs in their natural nests, the exact  
281 age in days of the chick was unknown, whereas tarsus length was available for all  
282 treatments.

283

284 We examined whether within each call type, there were changes in call structure  
285 between host environments. For each call type, the following begging call  
286 parameters were extracted from each recording: minimum frequency, maximum  
287 frequency, centre frequency, peak frequency, frequency bandwidth, call duration,  
288 average entropy, and energy. These are widely used in the literature to characterise  
289 begging sounds (Anderson et al., 2009; Butchart et al., 2003; Langmore et al., 2008).  
290 For each recording, ten sequential call notes in a bout of begging were selected and  
291 call parameters extracted. Call notes were not selected if they overlapped with  
292 interfering background noises, or if they were incomplete calls. The relationship  
293 between the call types was visualised using linear discriminant function analysis with

1  
2  
3 294 the R package MASS (Venables and Ripley, 2002). We calculated call rate by  
4  
5 295 dividing the dividing the number of calls in the bout by the duration of the bout.  
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10 297 Two approaches were used to compare the structure of each call type between pin-  
11  
12 298 tailed whydahs raised in common waxbill nests, and those raised in blue waxbill  
13  
14 299 nests. First, a series of linear mixed models were constructed, with each call  
15  
16 300 parameter as a separate response variable. Host environment and crop size were  
17  
18 301 fitted as fixed effects and individual chick identity as a random effect. Crop size was  
19  
20 302 used as a proxy for chick hunger. *When estrildid finch nestlings are fed, they store*  
21  
22 303 *their seed in the crop before passing it on to the stomach. By measuring the amount*  
23  
24 304 *of food stored in the crop, we could assess how much the chick had recently been*  
25  
26 305 *fed in a non-invasive manner. Crops were scored as 0 (empty), 1 (trace amounts, <*  
27  
28 306 *c. 20 seeds, and with no bulge in crop), 2 (> c. 20 seeds and with slight bulge) or 3*  
29  
30 307 *(> c. 50 seeds and with large bulge). We controlled for multiple testing using*  
31  
32 308 Bonferroni correction (Dunn, 1961). Second, we carried out a logistic regression  
33  
34 309 analysis using the R package nnet (Venables and Ripley, 2002), allowing all call  
35  
36 310 parameters to be considered at once.  
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## 47 **Results**

### 48 49 50 312 **1) Do hosts reject foreign eggs?**

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52  
53 313 Of 78 foreign eggs placed in recipient blue waxbill nests (mean blue waxbill egg size  
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55 314 in recipient clutches = 14.5 x 10.9 mm, n = 364), none was rejected. Transferred  
56  
57 315 eggs comprised 35 common waxbill (mean size = 12.9 x 10.0 mm), 11 pin-tailed  
58  
59 316 whydah (mean size = 15.6 x 11.3 mm) and 32 blue waxbill eggs (mean size = 14.5 x



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3 317 10.9 mm). Therefore, we found no evidence that blue waxbills reject eggs of other  
4  
5 318 estrildid or *Vidua* species, or those laid by another blue waxbill female. Eggs of all  
6  
7 319 three species were immaculate white, so the only visual cue for discrimination could  
8  
9 320 have been size, which appears not to have been used.  
10  
11

12 321

15  
16 322 **2) Do host parents discriminate against foreign chicks and, if so, is this**  
17  
18 323 **through actively removing them from the nest or feeding them less food?**  
19

20  
21 324 **Are foreign chicks rejected?**  
22

23  
24 325 Of the 78 foreign eggs and 16 newly-hatched chicks transferred to blue waxbill  
25  
26 326 nests, 38 were common waxbill, 21 pin-tailed whydah and 35 blue waxbill  
27  
28 327 eggs/chicks. None was confirmed to have been subsequently removed from the nest  
29  
30 328 at the chick stage by the host parents while the chick was still alive. Removals of  
31  
32 329 chicks from the nest seemed to happen after the chick had died in the nest, as  
33  
34 330 supported by observations at two nests in which a dead common waxbill chick was  
35  
36 331 observed in the experimental nest in the morning, and was no longer present in the  
37  
38 332 afternoon. The removal of the dead chick at one of these nests was captured on a  
39  
40 333 trail camera. Therefore, we found no evidence of active chick rejection by blue  
41  
42 334 waxbill parents of chicks of other estrildid or *Vidua* species, or of chicks from other  
43  
44 335 blue waxbill nests.  
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51  
52 337 **Do foreign chicks survive worse than host chicks?**  
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54  
55 338 Of the 94 transfers of pin-tailed whydahs ( $n = 21$ ), blue waxbills ( $n = 35$ ) and  
56  
57 339 common waxbills ( $n = 38$ ) to blue waxbill nests, the selective death of the transferred  
58  
59 340 chick occurred on 45 occasions. In the remaining 49 cases, either the whole nest  
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3 341 was destroyed (e.g. by a predator) or the chick fledged, and so data were right-  
4  
5 342 censored. The full model explained the data significantly better than the null model  
6  
7 343 (chi-squared = 35.2, df = 4,  $p < 0.001$ ), suggesting that, among the predictor  
8  
9 344 variables, at least one has a significant effect on chick survival. Of these predictor  
10  
11 345 variables, only chick species was found to significantly increase the explanatory  
12  
13 346 power of the model (chi-squared = 34.2, df = 2,  $p < 0.001$ ). This suggests that chick  
14  
15 347 survival in blue waxbill nests depended strongly on the species of chick transferred.  
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19 348

20  
21 349 When survival of the different transferred nestlings was compared, blue waxbill  
22  
23 350 nestlings were found to survive significantly better than either pin-tailed whydah  
24  
25 351 (survival analysis,  $Z = 3.64$ ,  $p < 0.001$ ) or common waxbill (survival analysis,  $Z =$   
26  
27 352  $4.71$ ,  $p < 0.001$ ) nestlings. Survival of transferred pin-tailed whydah nestlings was  
28  
29 353 not significantly different from that of common waxbill nestlings (survival analysis,  $Z$   
30  
31 354  $= 1.25$ ,  $p > 0.2$ ) (Figure 2).  
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35 355

### 36 356 **Are foreign chicks fed less food than host chicks?**

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41 357 Median crop size during the first 7 days of life differed significantly between the three  
42  
43 358 species of transferred nestlings (Kruskal-Wallis, chi-squared = 14.23, df = 2,  $p <$   
44  
45 359  $0.001$ , Figure 3). Blue waxbills had significantly higher median crop scores than  
46  
47 360 common waxbills (Dunn post-hoc test,  $Z = 3.12$ , adjusted p-value  $< 0.01$ ) and pin-  
48  
49 361 tailed whydahs (Dunn post-hoc test,  $Z = 3.04$ , adjusted p-value  $< 0.01$ ). There was  
50  
51 362 no evidence that median crop size differed between pin-tailed whydah and common  
52  
53 363 waxbill nestlings (Dunn post-hoc test,  $Z = 0.528$ , adjusted p-value  $> 0.8$ ) (Figure 3).  
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3 365 When crop size was modelled as an ordinal response variable, a mixed-effects  
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5 366 model with both chick species and number of nestmates as fixed effects (ordinal  
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7 367 mixed-effect model, AIC = 625.77) was found to have a poorer fit than one with only  
8  
9 368 chick species as a co-variate (ordinal mixed-effect model, AIC = 523.30), so number  
10  
11 369 of nestmates was removed as a co-variate. Crop scores differed significantly  
12  
13 370 between transferred blue waxbill and pin-tailed whydah nestlings (ordinal mixed-  
14  
15 371 effect model,  $Z = 2.62$ ,  $p < 0.01$ ) and between blue waxbill and common waxbill  
16  
17 372 nestlings (ordinal mixed-effect model,  $Z = 2.10$ ,  $p < 0.05$ ). However, there was no  
18  
19 373 significant difference between the crop scores of pin-tailed whydahs and common  
20  
21 374 waxbills (ordinal mixed-effect model,  $Z = 1.15$ ,  $p > 0.2$ ). The result was the same  
22  
23 375 regardless of whether the first six or eight days of development were considered  
24  
25 376 (Table S1). The median crop size of chicks over the first 7 days of life was strongly  
26  
27 377 positively associated with nestling survival (survival analysis,  $Z = -3.667$ ,  $p < 0.001$ ).  
28  
29 378 In summary, crop size data suggest that heterospecific chicks were fed less than  
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31 379 conspecific chicks, and that this negatively influenced their survival.  
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41 **381 Are pin-tailed whydahs and common waxbills fed less in novel host nest than**  
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43 **382 in natural nest?**  
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46 383 The crop scores of both pin-tailed whydahs (ordinal mixed-effect model,  $Z = 1.99$ ,  $p$   
47  
48 384  $< 0.05$ ) and common waxbills (ordinal mixed-effect model,  $Z = 3.18$ ,  $p < 0.01$ ) were  
49  
50 385 lower when chicks were raised in blue waxbill nests than when raised in their own  
51  
52 386 natural (common waxbill) nests. This suggests that there is a cost to host-switching  
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54 387 in that foreign chicks are fed less than they would be in their own natural nest.  
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3 389 **3) Do different potential host species feed their young different foods, thus**  
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5 390 **providing a barrier to colonisation?**  
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9 391 For nestlings of all eight locally-occurring estrildid species that were sampled,  
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11 392 grasses from the subfamily Panicoideae dominated their diet, accounting for over  
12  
13 393 99% of reads in each (Table S2). However, despite their diet contents appearing to  
14  
15 394 overlap extensively in Figure S2, there was a significant difference in the diet  
16  
17 395 composition at the subfamily level between chicks in common waxbill and blue  
18  
19 396 waxbill nests (pseudo  $F = 5.347$ ,  $p = 0.012$ , Figure S2). Chicks in common waxbill  
20  
21 397 nests were fed an even higher proportion of seeds from the subfamily Panicoideae  
22  
23 398 (99.9%) than were chicks in blue waxbill nests (99.6%). However, despite this  
24  
25 399 difference being statistically significant at the 5% level, it is unlikely to be biologically  
26  
27 400 meaningful given that both percentages are extremely high. Therefore, at least at the  
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29 401 subfamily level, the nestling diet of the estrildid finches occurring at the study site  
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31 402 seems remarkably homogeneous.  
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40 404 **4) Can parasitic nestlings alter their begging calls to match those of their novel**  
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42 405 **host?**  
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45 406 **How many types of begging calls do nestling pin-tailed whydahs make?**  
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48 407 Pin-tailed whydah nestlings produced four distinct begging call types over the course  
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50 408 of development in both common waxbill and blue waxbill nests: 1) short very high-  
51  
52 409 pitched call given singly; 2) high-pitched calls given in quick succession; 3) a  
53  
54 410 repeated mid-level call given in mid to late development; 4) a double call with two  
55  
56 411 components, 4a and 4b. The two components usually come sequentially, with a  
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58 412 short first note (call type 4a) immediately followed by a longer second note (call type  
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3 413 4b). We refer to call type 4 as “we-chee”, where “we” is 4a and “chee” is 4b (Figure  
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5 414 S4).

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7 415  
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9  
10 416 Discriminant function analysis was used to plot the relationships of the different call  
11  
12 417 types to one another (Figure S1). Linear discriminant 1 (LD1) explains 66.7% of the  
13  
14 418 variation in these parameters, and LD2 explains a further 22.6% of the variation. Call  
15  
16 419 duration made the largest contribution to the loadings of LD1 and LD2. Type 3 calls  
17  
18 420 overlap with type 4a calls on the graph of LD1 versus LD2 (Figure S1), suggesting  
19  
20 421 that they are very similar. The only distinction is that call type 4a is given before 4b in  
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22 422 a quick two-note alternating pattern (“we-chee” call), whereas call type 3 is given  
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24 423 singly or in rapid succession.

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31 425 **Does host environment influence the developmental stage at which pin-tailed**  
32  
33 426 **whydah nestlings make their different call types?**

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36 427 The four different call types tended to be produced at different stages in chick  
37  
38 428 development. When raised in their natural host’s nest (common waxbill), pin-tailed  
39  
40 429 whydahs make call type 1 during the first few days of development, start making call  
41  
42 430 type 3 during mid-development, then incorporate call type 2 and, in late  
43  
44 431 development, call type 4 (Figure S3). However, the developmental stages at which  
45  
46 432 call types were produced differed depending on whether the pin-tailed whydah was  
47  
48 433 raised in a common waxbill or a blue waxbill nest (Figure S3). Using tarsus size as a  
49  
50 434 proxy for developmental stage, call type 1 was produced by younger pin-tailed  
51  
52 435 whydah nestlings when raised in blue waxbill nests compared to when raised in  
53  
54 436 common waxbill nests (linear mixed model,  $t = -3.09$ ,  $p < 0.005$ ), as was call type 2  
55  
56 437 (linear mixed model,  $t = -3.87$ ,  $p < 0.001$ ). There was no significant difference in the  
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3 438 tarsus lengths of pin-tailed whydah chicks producing call type 3 (linear mixed model,  
4  
5 439  $t = -0.157$ ,  $p > 0.8$ ) or call type 4 (linear mixed model,  $t = -1.587$ ,  $p > 0.1$ ) when raised  
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7 440 in blue waxbill or common waxbill nests.  
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13 442 **Does host environment alter the structure and rate of begging calls in pin-**  
14  
15 443 **tailed whydahs?**

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17  
18 444 For each begging call type produced by nestling pin-tailed whydahs, we analysed  
19  
20 445 whether the call parameters differed depending on the host environment (Table S3).  
21  
22 446 Call types 2 and 3 showed no significant difference among host environments in any  
23  
24 447 of the eight call parameters. When pin-tailed whydahs were raised by blue waxbills,  
25  
26 448 frequency bandwidth was larger for call types 1, 4a and 4b. Their call duration was  
27  
28 449 longer for call type 4a, while peak frequency and centre frequency were higher for  
29  
30 450 call type 1. Finally, minimum frequency was lower in call types 4a and 4b, and  
31  
32 451 maximum frequency higher in call types 1 and 4b.  
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39 453 Crop size was found to influence some call parameters (Table S4), so the model was  
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41 454 re-run with crop size as a co-variate. For call type 4a, host environment still  
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43 455 influenced frequency bandwidth (chi-squared = 63.4,  $p < 0.001$ ), call duration (chi-  
44  
45 456 squared = 70.8,  $p < 0.001$ ), minimum (chi-squared = 8.68,  $p < 0.01$ ) and maximum  
46  
47 457 (chi-squared = 56.1,  $p < 0.001$ ) frequencies even when statistically controlling for  
48  
49 458 crop size. Similarly, for call type 4b, host environment still had a significant effect on  
50  
51 459 frequency bandwidth (chi-squared = 198,  $p < 0.001$ ), and minimum (28.1,  $p < 0.001$ )  
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53 460 and maximum frequencies (chi-squared = 182,  $p < 0.001$ ) after statistically  
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55 461 controlling for crop size.  
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3 463 Call rate did not differ between pin-tailed whydahs raised in nests of their natural  
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5 464 host, the common waxbill, and those raised in blue waxbill nests (chi-squared = 2.35,  
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7 465  $p = 0.125$ ).  
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11  
12 467 In summary, an experimentally induced shift in host environment did not trigger the  
13  
14 468 production of new call types, but was associated with slight shifts in the  
15  
16 469 developmental stage at which each call type was used.  
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## 20 21 **Discussion**

### 22 23 24 470 ***Discrimination against non-mimetic chicks, without rejection, by host parents***

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27 471 We found that estrildid host parents discriminate against non-mimetic chicks by  
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29 472 feeding them less food, but not rejecting them outright. This has important  
30  
31 473 implications for patterns of host colonisation, speciation and the evolution of mimicry.  
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33 474 If host parents evicted any chick that did not look like or sound like its own, it would  
34  
35 475 be difficult for *Vidua* to persist in a new host environment unless, by chance, the new  
36  
37 476 host young resembled the *Vidua* nestling. However, foster parents discriminated  
38  
39 477 against odd chicks only by providing them with less food. If sufficient parasitic  
40  
41 478 nestlings nevertheless survive, and return to parasitise the same new host species  
42  
43 479 when they are adult, then, over multiple generations, selection can incrementally  
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45 480 drive convergence of the parasitic chick's begging displays on those of the new  
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47 481 host's young, or vice versa (see Hauber and Kilner, 2007).  
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55 483 This result, of reduced survival in non-mimetic chicks, complements a study by  
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57 484 Schuetz (2005a). He observed that common waxbill nestlings with slight  
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59 485 manipulations to mouth marking colour grew less well than unmanipulated ones, but  
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3 486 did not find that these manipulations affected chick survival. We show, through  
4  
5 487 cross-fostering experiments in the field, that when there is greater phenotypic  
6  
7 488 divergence between parasite and host nestlings, as is found between pin-tailed  
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9 489 whydah and blue waxbill nestlings, this can generate more extreme selection directly  
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11  
12 490 affecting nestling survival.  
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16  
17 492 Discrimination against non-mimetic chicks occurred regardless of whether the  
18  
19 493 transferred chick was raised alongside host young, or on its own. This implies that  
20  
21 494 blue waxbill parents have an internal template of what their own chicks should look  
22  
23 495 or sound like, rather than only discriminating against the most odd-looking chick  
24  
25 496 within the current brood. This internal template could be learned by host parents or  
26  
27 497 else be innate. If learned, first-time breeders should be less discriminating against  
28  
29 498 mismatching chicks than birds that have had several broods (Langmore et al., 2009;  
30  
31 499 Lotem et al., 1995; Noh et al., 2018). This would suggest that any environmental  
32  
33 500 factors increasing the proportion of first-time breeders in the population of a potential  
34  
35 501 host would increase the likelihood of successful host colonisation by *Vidua*, as the  
36  
37 502 average levels of discrimination against mismatching chicks would be lower. A good  
38  
39 503 breeding season the previous year could result in large numbers of first-time  
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41 504 breeders laying clutches the following year, providing relatively benign conditions for  
42  
43 505 host switches. This would increase the likelihood of several *Vidua* individuals  
44  
45 506 simultaneously colonising the new host, making it more likely that the offspring will  
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47 507 grow up to find a mate raised by the same host.  
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3 509 ***Nestling diet as a potential barrier to host colonisation***  
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6 510 The nestling diets of all eight species of estrildid finch sampled at the study site in  
7  
8 511 Zambia were very similar. The plant component of the diet of all species was  
9  
10 512 dominated by grass seeds in the subfamily Panicoideae. Our findings are consistent  
11  
12 513 with previous work in other estrildids which suggests that most members of this  
13  
14 514 family feed themselves and their young almost exclusively on grass seeds (Morton  
15  
16 515 and Davies, 1983; Payne, 2010a; Zann and Straw, 1983), although the degree of  
17  
18 516 dietary preference for particular grass species seems to vary among species  
19  
20 517 (Schöpfer, 1989; Zann and Straw, 1984). The resolution of the DNA barcoding  
21  
22 518 approach only allowed us to identify crop samples to the subfamily level, and it is  
23  
24 519 possible that biologically-relevant variation in diet exists at finer taxonomic scales. As  
25  
26 520 a greater number of African grass species become barcoded, the resolution of this  
27  
28 521 approach will likely increase.  
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36 523 The apparent uniformity of nestling diets among estrildid finch species suggests that  
37  
38 524 adaptation to novel diets is unlikely to be a major barrier in the colonisation of new  
39  
40 525 estrildid hosts by *Vidua*. Instead, it seems that the major barrier to colonising new  
41  
42 526 hosts for parasites is ensuring they get fed the right *amount* of food from host  
43  
44 527 parents, rather than securing the right *type* of food. This implies that any conditions  
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46 528 increasing food abundance may increase the probability of simultaneous successful  
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48 529 colonisations of new hosts.  
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3 531 ***Can Vidua plastically shift their begging calls depending on host***  
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5 532 ***environment?***  
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9 533 Transfer to a new host environment did not trigger the development of novel begging  
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11 534 call types, since all pin-tailed whydah begging call types were produced both by pin-  
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13 535 tailed whydahs raised in common waxbill nests and those raised in blue waxbill  
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15 536 nests. However, there were slight changes in the developmental stage at which each  
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17 537 call type was used depending on which host was raising them. A possible  
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19 538 interpretation is that the pin-tailed whydah chicks chose, from an innate repertoire of  
20  
21 539 call types, to use the call type which most resembled that of their host and was  
22  
23 540 therefore most effective at stimulating parents to feed them. Such “social shaping” of  
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25 541 begging calls has been reported from another brood parasite, the Horsfield’s bronze-  
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27 542 cuckoo (*Chalcites basalis*) (Langmore et al., 2008).  
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32 543  
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34 544 The absence of large-scale plastic shifts in begging calls by nestling *Vidua*  
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36 545 depending on host environment is consistent with theory put forward by Jamie and  
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38 546 Kilner (2017). We suggested that such plasticity is likely to be a characteristic of  
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40 547 generalist brood parasites that exploit a wide variety of hosts. By contrast, in brood-  
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42 548 parasitic species that are highly specialized on a single or small number of host  
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44 549 species, as in the *Vidua* finches, begging call structure is more likely to be  
45  
46 550 determined genetically. Innate, mimetic begging calls have been found in the  
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48 551 nestlings of one other specialist brood-parasitic species, the screaming cowbird  
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50 552 (*Molothrus rufoaxillaris*), which retained their specialist begging call when transferred  
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52 553 to a non-natural host nest (De Mársico et al., 2012; Ripari et al., 2018).  
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3 555 The lack of adaptive plasticity in begging calls in *Vidua* may promote host  
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5 556 specialisation, and therefore speciation. *Vidua* begging calls seem to be primarily a  
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7 557 genetic adaptation to hosts and the lack of plasticity prevents a single *Vidua* species  
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9 558 from successfully exploiting multiple hosts. Instead, genetic adaptation in a suite of  
10  
11 559 begging display related traits is required. Thus, genetic adaptations to a single host  
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13 560 simultaneously make the nestling less good at exploiting another host species with  
14  
15 561 different begging displays, promoting specialisation. Therefore, even though  
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17 562 adaptive plasticity in begging calls would facilitate the initial survival of *Vidua*  
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19 563 nestlings in a novel host environment, thus promoting host colonisation and  
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21 564 speciation, it would potentially also constrain diversification by relaxing the  
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23 565 evolutionary pressure for host-specific genetic adaptation.  
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### 32 567 ***An integrated picture of host colonisation and speciation***

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35 568 Taken together, these findings present the following picture of the *Vidua* radiation.  
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37 569 As nestlings, many *Vidua* mimic the mouth markings, begging calls and head  
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39 570 movements of their hosts (Jamie et al., 2020; Payne, 2005). These mimetic traits  
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41 571 develop primarily using genetic cues, with only slight changes depending on host  
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43 572 environment.  
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49 574 A key barrier to host colonisation, and therefore speciation, for *Vidua* is persuading  
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51 575 host parents to feed them adequate quantities of food rather than the right *type* of  
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53 576 food. A strong phylogenetic signal in begging display traits (Payne, 2005) potentially  
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55 577 explains why successful new colonisations tend to involve species that are closely  
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57 578 related to the ancestral host – “clade-limited colonisation” (Sorenson et al., 2004).  
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3 579 We might hypothesise that certain clades that are seemingly suitable for colonisation  
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5 580 by the parasite have not been colonised because their begging traits are too  
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7 581 dissimilar, and the initial fitness barrier to parasitism is therefore too high. For  
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9  
10 582 example, no genera with a bar pattern rather than spots on the upper palate are  
11  
12 583 currently known to be parasitised. *Vidua* seem not to have evolved to mimic this  
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14 584 upper palate bar, and so it potentially provides a fail-safe cue for estrildid parents of  
15  
16 585 species possessing this trait to use in provisioning decisions. Similarly, no *Vidua*  
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18 586 have evolved the blue upper palate colour found in *Uraeginthus* waxbills, again  
19  
20 587 potentially explaining why none of these species has successfully been colonised. In  
21  
22 588 addition to nestling adaptations, habitat seems to provide an important filter limiting  
23  
24 589 which estrildid finches become parasitised by *Vidua* finches (Péron et al., 2016). For  
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26 590 example, none of the forest-living estrildids are regularly parasitised.  
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33 592 Given the large costs to a parasite of laying their eggs in the nest of the “wrong” host  
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35 593 species, it is reasonable to ask why *Vidua* parents still occasionally do this (Hockey  
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37 594 et al., 2005; Tarboton, 2011, GAJ pers obs). Is it a purely maladaptive consequence  
38  
39 595 of mistakes by parasite parents, or could there be an adaptive explanation? Laying in  
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41 596 the “wrong” host species’ nest is analogous to colonisation of a “sink” habitat whose  
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43 597 population is maintained by immigration from “source” habitats rather than by  
44  
45 598 successful reproduction *in situ* (Holt, 1997; Pulliam, 1988). A host is successfully  
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47 599 colonised once it transitions from being a “sink” to a “source” environment for the  
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49 600 *Vidua*. This occurs when the population of *Vidua* specialising on that host is  
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51 601 maintained by successful reproduction within this species’ nest, rather than from  
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53 602 accidental laying by parasite parents primarily exploiting a different host species.  
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3 604 Three hypotheses put forward for why organisms may persistently move into “sink”  
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5 605 environments are: 1) all “source” habitats are saturated or unavailable, 2) organisms  
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7 606 are unable to distinguish between “sink” and “source” habitats and 3) “source”  
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9 607 environments have unstable local population dynamics (Holt, 1997).

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14 609 Hypothesis 1 could help explain why *Vidua* parents sometimes lay eggs in the wrong  
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16 610 nest. Host population dynamics fluctuate over space and time, varying with  
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18 611 environmental variables like rainfall (Péron et al., 2016). Particularly towards the  
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20 612 beginning or end of the breeding season, the primary host may not be nesting in  
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22 613 abundance and the *Vidua* parent may fail to find an appropriate nest at the correct  
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24 614 stage. In such circumstances, she may be forced to make the best of a bad job and  
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26 615 lay her egg in the nest of a different host with a much lower likelihood of chick  
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28 616 survival. Similarly, hypothesis 2 is also likely to apply in the *Vidua* system. All *Vidua*  
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30 617 host species are in a single family, the Estrildidae, and many members of the same  
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32 618 genus are often parasitised by different *Vidua* species. *Vidua* females may  
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34 619 accidentally lay their eggs in the nest of the wrong species as they may have similar  
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36 620 adult appearance, calls and nest architecture. Given that closely related estrildid  
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38 621 species also have more similar nestling mouth markings (Jamie, 2017a; Payne,  
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40 622 2005), it may be that such “mistakes” are the pre-cursors to the successful  
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42 623 colonisation of new hosts and speciation.

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47 625 It is not clear whether hypothesis 3 applies to the *Vidua* system. This hypothesis  
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49 626 proposes that “sink” populations are maintained due to temporal fluctuations in the  
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51 627 relative fitness of “source” populations compared to “sink” populations (Holt, 1997).  
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53 628 In *Vidua* the source of the selection pressure against the colonisation of new hosts is  
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3 629 discrimination against odd chicks by host parents. Two factors that could cause host  
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5 630 discrimination levels to fluctuate are food abundance and how experienced host  
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7 631 parents are. However, there are currently no studies on how parental discrimination  
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9 632 varies with environmental factors or how an internal template of chick appearance  
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11 633 develops in estrildid finches.  
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17 635 In conclusion, taken together with previous studies, our results suggest that the  
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19 636 extent of this radiation of parasites can be understood through the complexity of host  
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21 637 begging displays, the strong phylogenetic conservatism of host begging displays, the  
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23 638 discrimination by estrildid parents against odd chicks, and the lack of adaptive  
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25 639 plasticity in begging display traits by *Vidua*, all in combination with habitat filters. We  
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27 640 suggest that these factors have limited diversification in this clade, despite the  
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29 641 availability of seemingly suitable, yet unparasitised, estrildid species overlapping  
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31 642 geographically with *Vidua*. More generally, our results suggest that the extent of  
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33 643 radiation in parasite lineages is determined by the extent of diversity in the host traits  
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35 644 that determine parasite fitness, and the fitness consequences of parasites being  
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37 645 maladapted to a new host.  
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#### 43 44 647 **Data accessibility statement**

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47 648 Raw data and associated R scripts will be uploaded to Dryad on acceptance of the  
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49 649 manuscript.  
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## References

- 650  
651  
652 Anderson MG, Ross HA, Brunton DH, Hauber M, 2009. Begging call matching  
653 between a specialist brood parasite and its host: a comparative approach to  
654 detect coevolution. *Biol J Linn Soc* 98:208-216.
- 655 Buchanan K, Burt de Perera T, Carere C, Carter T, Hailey A, Hubrecht R, Jennings  
656 D, Metcalfe N, Pitcher T, Peron F, Sneddon L, Sherwing C, Talling J, Thomas  
657 R, Thompson M, 2012. Guidelines for the treatment of animals in behavioural  
658 research and teaching. *Anim Behav* 83:301-309.
- 659 Bush GL, 1969. Sympatric host race formation and speciation in frugivorous flies of  
660 the genus *Rhagoletis*. *Evolution* 23:237-251.
- 661 Butchart SHM, Kilner RM, Fuisz T, Davies NB, 2003. Differences in the nestling  
662 begging calls of hosts and host-races of the common cuckoo, *Cuculus*  
663 *canorus*. *Anim Behav* 65:345-354. doi: 10.1006/anbe.2003.2066.
- 664 Christensen RHB, 2015. ordinal - Regression Models for Ordinal Data. R package  
665 version 2015.6-28.
- 666 Cox DR, 1972. Regression models and life-tables. *J Roy Stat Soc Ser B (Stat*  
667 *Method)* 34:187-220.
- 668 Craine JM, Towne EG, Miller M, Fierer N, 2015. Climatic warming and the future of  
669 bison as grazers. *Sci Rep* 5:16738. doi: 10.1038/srep16738.
- 670 De Mársico MC, Gantchoff MG, Reboreda JC, 2012. Host-parasite coevolution  
671 beyond the nestling stage? Mimicry of host fledglings by the specialist  
672 screaming cowbird. *Proc R Soc B* 279:3401-3408. doi:  
673 10.1098/rspb.2012.0612.
- 674 Author. 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R  
675 package version 1.3.4. <https://cran.r-project.org/package=dunn.test>.
- 676 Dunn OJ, 1961. Multiple comparisons among means. *Journal of the American*  
677 *Statistical Association* 56:52-64.
- 678 Filchak KE, Roethele JB, Feder JL, 2000. Natural selection and sympatric  
679 divergence in the apple maggot *Rhagoletis pomonella*. *Nature* 407:739-742.  
680 doi: 10.1038/35037578.
- 681 Fordyce JA, 2010. Host shifts and evolutionary radiations of butterflies. *Proc R Soc B*  
682 277:3735-3743. doi: 10.1098/rspb.2010.0211.
- 683 Hardy NB, Otto SP, 2014. Specialization and generalization in the diversification of  
684 phytophagous insects: tests of the musical chairs and oscillation hypotheses.  
685 *Proc R Soc B* 281. doi: 10.1098/rspb.2013.2960.

- 1  
2  
3 686 Hauber ME, Kilner RM, 2007. Coevolution, communication, and host chick mimicry  
4 687 in parasitic finches: who mimics whom? Behav Ecol Sociobiol 61:497-503.  
5 688 doi: 10.1007/s00265-006-0291-0.  
6  
7  
8 689 Hockey PAR, Dean WRJ, Ryan PG, 2005. Roberts Birds of Southern Africa, VIIIth  
9 690 ed. Cape Town: The Trustees of the John Voelcker Bird Book Fund.  
10  
11 691 Holt RD, 1997. On the evolutionary stability of sink populations. Evol Ecol 11:723-  
12 692 731.  
13  
14 693 Jamie GA, 2017a. Mimicry and speciation in the parasitic finches of Africa [PhD].  
15 694 Cambridge: University of Cambridge.  
16  
17 695 Jamie GA, 2017b. Signals, cues and the nature of mimicry. Proc R Soc B  
18 696 284:20162080. doi: 10.1098/rspb.2016.2080.  
19  
20  
21 697 Jamie GA, Kilner RM, 2017. Begging Call Mimicry by Brood Parasite Nestlings:  
22 698 Adaptation, Manipulation and Development. In: Soler M, editor. Avian Brood  
23 699 Parasitism: Behaviour, Ecology, Evolution and Coevolution: Springer  
24 700 Publishing Company. p. 517-538.  
25  
26 701 Jamie GA, Van Belleghem SM, Hogan B, Hamama S, Moya C, Troscianko J,  
27 702 Stoddard MC, Kilner RM, Spottiswoode CN, 2020. Multimodal mimicry of  
28 703 hosts in a radiation of parasitic finches. Evolution. doi:  
29 704 <https://doi.org/10.1111/evo.14057>.  
30  
31  
32 705 Janz N, Nylin S, Wahlberg N, 2006. Diversity begets diversity: host expansions and  
33 706 the diversification of plant-feeding insects. BMC Evol Biol 6:4. doi:  
34 707 10.1186/1471-2148-6-4.  
35  
36 708 Langmore NE, Cockburn A, Russell AF, Kilner RM, 2009. Flexible cuckoo chick-  
37 709 rejection rules in the superb fairy-wren. Behav Ecol 20:978-984. doi:  
38 710 10.1093/beheco/arp086.  
39  
40  
41 711 Langmore NE, Maurer G, Adcock GJ, Kilner RM, 2008. Socially acquired host-  
42 712 specific mimicry and the evolution of host races in Horsfield's bronze-cuckoo  
43 713 *Chalcites basalis*. Evolution 62:1689-1699. doi: 10.1111/j.1558-  
44 714 5646.2008.00405.x.  
45  
46 715 Lotem A, Nakamura H, Zahavi A, 1995. Constraints on egg discrimination and  
47 716 cuckoo-host co-evolution. Anim Behav 49:1185-1209.  
48  
49  
50 717 McPheron BA, Smith DC, Berlocher SH, 1988. Genetic differences between host  
51 718 races of *Rhagoletis pomonella*. Nature 336:64-66.  
52  
53 719 Mills M, 2011. Introducing Survival and Event History Analysis. London: Sage.  
54  
55 720 Author. 2012. KMSurv: Data sets from Klein and Moeschberger (1997), Survival  
56 721 Analysis, R package version 0.1-5.  
57  
58  
59  
60



- 1  
2  
3 722 Morton SR, Davies PH, 1983. Food of the zebra finch (*Poephila guttata*), and an  
4 723 examination of granivory in birds of the Australian arid zone. *Aust J Ecol*  
5 724 8:235-243.
- 7 725 Neunzig R, 1929. Zum Brutparasitismus der Viduinen. *Journal für Ornithologie* 77:1-  
8 726 22.
- 11 727 Nicolai J, 1964. Der Brutparasitismus der Viduinae als ethologisches Problem. *Z*  
12 728 *Tierpsychol* 21:129-204.
- 14 729 Nicolai J, 1974. Mimicry in parasitic birds. *Sci Am* 231:92-98.
- 16 730 Nicolai J, 1989. Brutparasitismus der Glanzwitwe (*Vidua hypocherina*). *Journal für*  
17 731 *Ornithologie* 130:423-434.
- 19 732 Nicolai J, 1991. Vom Buntastrild und seinen Brutparasiten. *Gefiederte Welt* 115:369-  
20 733 371.
- 23 734 Noh HJ, Gloag R, Langmore NE, 2018. True recognition of nestlings by hosts selects  
24 735 for mimetic cuckoo chicks. *Proc R Soc B* 285. doi: 10.1098/rspb.2018.0726.
- 26 736 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR,  
27 737 O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H,  
28 738 2017. *vegan: Community Ecology Package*. R package version 2.4-3.
- 30 739 Payne RB, 2005. Nestling mouth markings and colors of old world finches  
31 740 Estrildidae: mimicry and coevolution of nesting finches and their *Vidua* brood  
32 741 parasites. *Miscellaneous Publications Museum of Zoology, University of*  
33 742 *Michigan* 194.
- 36 743 Payne RB, 2010a. Family Estrildidae (Waxbills). In: del Hoyo J, Elliot A, Christie DA,  
37 744 editors. *Handbook of the Birds of the World Vol 15 Weavers to New World*  
38 745 *Warblers* Barcelona: Lynx Edicions.
- 40 746 Payne RB, 2010b. Family Viduidae (Whydahs and Indigobirds). In: del Hoyo J, Elliot  
41 747 A, Christie DA, editors. *Handbook of the Birds of the World Vol 15 Weavers to*  
42 748 *New World Warblers* Barcelona: Lynx Edicions.
- 45 749 Payne RB, Payne LL, 2002. Begging for parental care from another species:  
46 750 specialization and generalization in brood-parasitic finches. In: Horn AG,  
47 751 Leonard ML, editors. *The evolution of begging: competition, cooperation &*  
48 752 *communication* Dordrecht, The Netherlands: Kluwer Academic Publishers.
- 50 753 Payne RB, Payne LL, Woods JL, 1998. Song learning in brood-parasitic indigobirds  
51 754 *Vidua chalybeata*: song mimicry of the host species. *Anim Behav* 55:1537-  
52 755 1553.
- 55 756 Payne RB, Payne LL, Woods JL, Sorenson MD, 2000. Imprinting and origin of  
56 757 parasite-host species associations in brood-parasitic indigobirds, *Vidua*  
57 758 *chalybeata*. *Anim Behav* 59:69-81.
- 59  
60

- 1  
2  
3 759 Payne RB, Woods JL, Payne LL, 2001. Parental care in estrildid finches:  
4 760 experimental tests of a model of *Vidua* brood parasitism. Anim Behav 62:473-  
5 761 483. doi: 10.1006/anbe.2001.1773.
- 7 762 Péron G, Altwegg R, Jamie GA, Spottiswoode CN, 2016. Coupled range dynamics of  
8 763 brood parasites and their hosts responding to climate and vegetation  
9 764 changes. J Anim Ecol 85:1191-1199. doi: 10.1111/1365-2656.12546.
- 11 765 Poulin R, Morand S, 2000. The diversity of parasites. The Quarterly Review of  
12 766 Biology 75:277-293.
- 14 767 Pullinam HR, 1988. Sources, sinks and population regulation. The American  
15 768 Naturalist 132:652-661.
- 17 769 Author. 2017. R: A language and environment for statistical computing. R  
20 770 Foundation for Statistical Computing. Vienna, Austria.
- 21 771 Ripari JMR, Ursino CA, Reboreda JC, De Mársico MC, 2018. Innate development of  
22 772 acoustic signals for host parent-offspring recognition in the brood-parasitic  
23 773 Screaming Cowbird *Molothrus rufoaxillaris*. Ibis. doi: 10.1111/ibi.12672.
- 25 774 Schluter D, 2000. The Ecology of Adaptive Radiations. New York, United States:  
26 775 Oxford University Press.
- 28 776 Schöpfer M, 1989. Feeding ecology of five sympatric species of grassfinches in  
29 777 southeastern Australia: LaTrobe University, Bundoora.
- 31 778 Schuetz JG, 2005a. Reduced growth but not survival of chicks with altered gape  
32 779 patterns: implications for the evolution of nestling similarity in a parasitic finch.  
33 780 Anim Behav 70:839-848. doi: 10.1016/j.anbehav.2005.01.007.
- 35 781 Schuetz JG, 2005b. Low survival of parasite chicks may result from their imperfect  
36 782 adaptation to hosts rather than expression of defenses against parasitism.  
37 783 Evolution 59, 9, 2017-2024
- 39 784 Sorenson MD, Balakrishnan C, Payne RB, 2004. Clade-limited colonization in brood  
40 785 parasitic finches (*Vidua* spp.). Syst Biol 53:140-153. doi:  
41 786 10.1080/10635150490265021.
- 43 787 Sorenson MD, Sefc KM, Payne RB, 2003. Speciation by host switch in brood  
44 788 parasitic indigobirds. Nature 424:928-931.
- 46 789 Stroud JT, Losos JB, 2016. Ecological opportunity and adaptive radiation. Annual  
47 790 Review of Ecology, Evolution, and Systematics 47:507-532. doi:  
48 791 10.1146/annurev-ecolsys-121415-032254.
- 50 792 Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, Vermet T,  
51 793 Corthier G, Brochmann C, Willerslev E, 2007. Power and limitations of the  
52 794 chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Res  
53 795 35:e14. doi: 10.1093/nar/gkl938.
- 55  
56  
57  
58  
59  
60

- 1  
2  
3 796 Tarboton W, 2011. Roberts Nests and Eggs of southern African birds. Cape Town,  
4 797 South Africa: The Trustees of the John Voelcker Bird Book Fund.  
5  
6 798 Author. 2015. A Package for Survival Analysis in S. Version 2.38.  
7  
8 799 Venables WN, Ripley BD, 2002. Modern applied statistics with S, Fourth Edition ed.  
9 800 New York: Springer.  
10  
11  
12 801 Willerslev E, Davison J, Moora M, Zobel M, Coissac E, Edwards ME, Lorenzen ED,  
13 802 Vestergård M, Gussarova G, Haile J, Craine J, Gielly L, Boessenkool S, Epp  
14 803 LS, Pearman PB, Cheddadi R, Murray D, Bråthen KA, Yoccoz N, Binney H,  
15 804 Cruaud C, Wincker P, Goslar T, Alsos IG, Bellemain E, Brysting AK, Elven R,  
16 805 Sønstebø JH, Murton J, Sher A, Rasmussen M, Rønn R, Mourier T, Cooper  
17 806 A, Austin J, Möller P, Froese D, Zazula G, Pompanon F, Rioux D, Niderkorn  
18 807 V, Tikhonov A, Savvinov G, Roberts RG, MacPhee RDE, Gilbert MTP, Kjær  
19 808 KH, Orlando L, Brochmann C, Taberlet P, 2014. Fifty thousand years of Arctic  
20 809 vegetation and megafaunal diet. *Nature* 506:47-51. doi: 10.1038/nature12921.  
21  
22  
23 810 Zann R, Straw B, 1983. A non-destructive method to determine the diet of seed-  
24 811 eating birds. *Emu* 84:40-41.  
25  
26 812 Zann R, Straw B, 1984. Feeding ecology and breeding of Zebra Finches in farmland  
27 813 in northern Victoria. *Australian Wildlife Research* 11:533-552.  
28  
29 814 Ziętara MS, Lumme J, 2002. Speciation by host switch and adaptive radiation in a  
30 815 fish parasite genus *Gyrodactylus*. *Evolution* 56:2445-2458.  
31  
32 816  
33  
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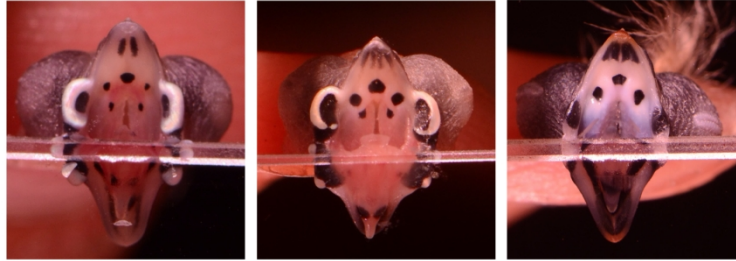


Figure 1. Mouth markings of newly-hatched nestling common waxbill (left), pin-tailed whydah (centre) and blue waxbill (right). These images show how different the visual stimulus provided by a blue waxbill nestling is compared to that of a pin-tailed whydah or common waxbill. Pin-tailed whydahs mimic the mouth pattern and colour of their natural host, common waxbill (Jamie et al. 2020). To obtain these photos, the nestling is held gently biting the edge of a prism such that the image of the inside of the mouth is projected onto the opposing flat surface of the prism (see Jamie et al. 2020 for detailed methods). The line across the center of the images is the edge of the prism.

338x190mm (150 x 150 DPI)

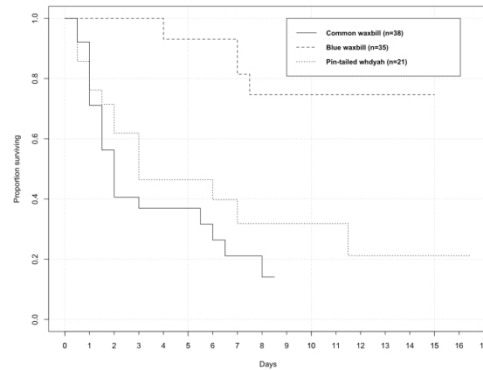


Figure 2. Survival curves for common waxbills (natural host), pin-tailed whydahs (parasite) and blue waxbills (novel host) transferred to Blue waxbill nests. Blue waxbills survived significantly longer in blue waxbill nests than either common waxbills (survival analysis,  $z = 4.709$ ,  $p < 10^{-5}$ ) or pin-tailed whydahs did (survival analysis,  $z = 3.642$ ,  $p < 0.001$ ). There was no significant difference in survival between common waxbills and pin-tailed whydahs (survival analysis,  $z = 1.247$ ,  $p > 0.2$ ).

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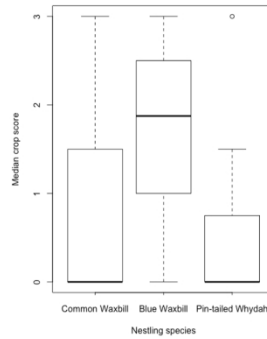


Figure 3. Median crop size from day 0 to day 7 for chicks that had been transferred to blue waxbill nests. Median crop size significantly differed between species (Kruskal-Wallis chi-squared = 14.2,  $p < 0.001$ ). Blue waxbills had significantly higher median crop scores than common waxbills (Dunn post-hoc test,  $Z = 3.12$ , adjusted  $p$ -value  $< 0.01$ ) and pin-tailed whydahs (Dunn post-hoc test,  $Z = 3.04$ , adjusted  $p$ -value  $< 0.01$ ). There was no evidence that median crop size differed between pin-tailed whydah and common waxbill nestlings (Dunn post-hoc test,  $Z = 0.528$ , adjusted  $p$ -value  $> 0.8$ ). Sample sizes: Blue Waxbill ( $n=33$ ), Common Waxbill ( $n=24$ ), Pin-tailed Whydah ( $n=12$ ).

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