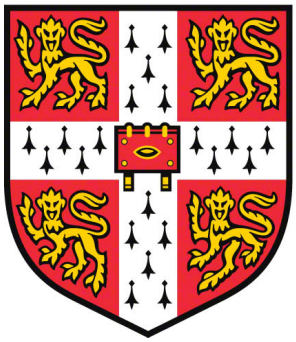

MIMICRY AND SPECIATION IN THE PARASITIC FINCHES OF AFRICA

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Declaration

I, Gabriel A. Jamie, confirm that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as specified in the text. The text is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as specified in the text. The text does not exceed 60,000 words.

Summary

Adaptive radiations have produced some of the most stunning showcases of diversity in the natural world. From the myriad of cichlid fish species that inhabit the lakes of east Africa to the beautifully varied vangas of Madagascar. But what determines the extent of these radiations? Why have some diversified to contain hundreds whereas others only tens of species? To answer these questions, we need to study the processes that limit the colonisation of new ecological niches and how colonisation generates reproductive isolation between lineages occupying different niches.

In this thesis, I study a radiation of brood-parasitic finch species, the indigobirds and whydahs (genus *Vidua*), that occur across Africa. *Vidua* finches consist of 19 mostly host-specific brood parasites, each laying their egg in a different species of host in the Estrildidae family. Host usage is the ecological niche varying between *Vidua* species and underpins this radiation of parasitic finches. Host colonisation is tightly linked to speciation in *Vidua* because of their remarkable capacity to imprint on their hosts, with mating traits and host preferences being strongly influenced by the host environment the finch grew up in. Additionally, *Vidua* possess host-specific adaptations to effectively exploit a host species. They mimick the appearance of their host's nestlings. Given this knowledge of *Vidua* biology, the challenge of explaining why the radiation has diversified to the extent it has simplifies to explaining why only some host species have been successfully colonised whereas others have not.

Following on from the introduction (Chapter 1), I begin by critically examining the logic with which mimicry in the natural world can be conceptually organized (Chapter 2). This creates a "mimicry landscape" in which to situate the mimetic adaptations of hosts exhibited by *Vidua.* The framework can be used to contrast and draw parallels between these and other mimetic adaptations present in the natural world. In Chapter 3, I review the literature on begging call mimicry and development across all avian brood parasite species. I outline the conditions under which we expect begging call mimicry to evolve, and when we expect it to develop primarily through genetic or environmental cues. This provides clear predictions for what we expect to occur in *Vidua* finches, which are tested in Chapters 4 and 5. In Chapter 4, I quantify the mimicry of host nestlings by *Vidua* in detail. I provide the first quantitative evidence that *Vidua* nestlings mimic the begging calls and show for the first time

that *Vidua* are imperfect mimics of their hosts. In Chapter 5, I simulate the colonisation of a new host by transferring *Vidua* eggs into the nest of a new host species. I monitor *Vidua* survival in the foreign host environment and test several hypotheses about what explains differences in chick survival. I find that *Vidua* survive poorly in the new nest environment and that they do not show adaptive plasticity in begging calls or head movements. This poor survival occurs despite there being minimal differences in the diets each host species feeds their young. Finally, in Chapter 6, I carry out a comparative analysis on the evolution of estrildid mouth markings. Estrildid finches are the hosts of *Vidua* and so provide the landscape of potential ecological niches that *Vidua* may colonise and adapt to. I demonstrate that the host family shows strong phylogenetic signal in mouth marking traits, and find no evidence that ecological factors such as light environment or predation pressure has shaped estrildid mouth marking evolution. I find no evidence that parasitism by *Vidua* has altered the mouth marking evolution of the hosts.

The work in this thesis highlights how difficult successfully colonising new hosts is for *Vidua* finches. This is because, unless the ancestral and new host have very similar begging displays, *Vidua* must mimic hosts in multiple traits (mouth markings, begging calls, head movements) to obtain sufficient amounts of food from host parents. Additionally, *Vidua* show no evidence of adaptive plasticity in traits such as begging calls and head movements which could otherwise have compensated for the lack of genetic adaptations to a new host immediately following colonisation. Overall, habitat filters, the complex and diverse begging displays of estrildid nestlings, the discriminatory behaviour of estrildid parents against mismatching chicks and the lack of adaptive plasticity in begging displays by *Vidua* together help explain why the *Vidua* radiation consists of only 19 species rather than many more or fewer. My research demonstrates the importance of combining experimental, comparative and theoretical approaches to understanding adaptive radiations in the natural world. By studying individual radiations in depth and situating them in a broader context we can develop general theories about the processes creating and limiting diversity in the natural world.

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Throughout my thesis I have been lucky enough to be supported and advised by many great people. Without them, the work in presented here could not have been accomplished. First, I thank my supervisor Claire Spottiswoode for being a wonderful mentor, colleague and friend. She has guided and encouraged me through the challenges of research and provided a gold standard to aspire towards as a scientist. I thank Rebecca Kilner for her wise advice and many stimulating discussions over the past years. Nick Davies and Chris Jiggins have provided ongoing support and deep insight that has greatly helped to improve the contents of this thesis. I am very fortunate to have such a fantastic advisory team.

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Contents

Chapter 1: *Introduction*

Chapter 1: Introduction

Evolutionary biology aims to understand the processes that generate, limit and diminish diversity in the natural world. Research on adaptive radiations can provide important insights into these processes. Adaptive radiations occur when lineages rapidly diversify as they adapt to different ecological niches (Schluter 2000). Classic examples include the Darwin's finches of the Galapagos (Grant and Grant 2008), the *Anolis* lizards of the Caribbean (Losos 2009), the cichlids of the East African lakes (Kornfield and Smith 2000), the honeycreepers of Hawaii (Pratt 2005) and the vangas of Madagascar (Reddy et al. 2012). In each of these, a single ancestor speciates into a myriad of forms, each occupying separate niches. Through comparative and experimental studies, we can both reconstruct and simulate key events that have shaped the evolutionary trees of these lineages. Detailed studies on recently-diverged populations are particularly useful because they allow us to bridge the gap between micro and macroevolution. If we study radiations across a wide enough range of contexts, we can develop general theories about the roles different evolutionary forces play under different circumstances.

The indigobirds and whydahs (genus *Vidua*) of sub-Saharan Africa provide a wonderful system in which to investigate the processes shaping adaptive radiations. The genus *Vidua* comprises 19 species of finch occurring across sub-Saharan Africa (Payne 1982; Payne 1998). All *Vidua* are obligate, inter-specific brood parasites (Payne and Bonan 2017b). This means that *Vidua* never raise their own offspring and instead deposit their eggs in the nest of another species. Obligate brood parasitism is thought to have evolved as a strategy in birds seven times: three times in the cuckoo family (Cuculidae) (Sorenson and Payne 2005), and once each in the honeyguides, cowbirds, ducks and parasitic finches (Sorenson and Payne 2001). *Vidua* do not kill their nest mates but are instead raised alongside their host nestmates (Payne 1973). In this respect they are similar to several other groups of avian brood parasites including the cowbirds, *Clamator* cuckoos and the Channel-billed Cuckoo (*Scythrops novaehollandiae*), but differ from the honeyguides (Spottiswoode and Koorevaar 2012) and the remaining parasitic cuckoo species (Davies 2000). Most *Vidua* finches are host-specific, each parasitising a different host species. However, a couple of species, the Cameroon Indigobird (*V. camarunensis*) (Payne et al. 2005) and the Pin-tailed Whydah, have been recorded to parasitise more than one host species (Hockey et al. 2005; Tarboton 2011).

All *Vidua* host species are in the estrildid finch family (Estrildidae) (Figures 1.1 and 1.2). Estrildid finches are a large group, containing around 140 species occurring in the Afrotropical as well as Oriental and Australasian biogeographic regions (Payne and Bonan 2017a), and are sister to the *Vidua* family (Viduidae) (Sorenson and Payne 2001). The two families share a common ancestor around 15–16 million years ago (Gomes et al. 2016). The nestlings of estrildid finches are highly ornamented and diverse, and the chicks of most species have a characteristic appearance. In particular, nestling estrildids have elaborate and species-specific patterns of spots and swellings inside their mouths, as well as varying among species in nestling skin colour and the distribution of natal down (Payne 2005b) (Figure 1.3). The mouth markings have been shown to be important in soliciting care from parents (Payne et al. 2001; Schuetz 2005b). *Vidua* species specialising on a given estrildid finch have evolved mouth markings that closely match those of their specific host (Neunzig 1929; Nicolai 1964; Payne 1982). Each potential host species therefore represents a distinct ecological niche that a parasite could colonise and adapt to.

The first evidence of brood parasitism by *Vidua* comes from Austin Roberts, writing in 1907 about the Pin-tailed Whydah (*V. macroura*) in South Africa (Roberts 1907). The first suggestions that the close match in mouth marking appearance between parasite and host represents adaptive convergence came from work done on birds in captivity by Rudolf Neunzig (Neunzig 1929). This work was extended to a greater variety of species by Jürgen Nicolai (Nicolai 1964; Nicolai 1969; Nicolai 1973). Due to uncertainty about the phylogenetic relationships between *Vidua* and estrildid finches, some authors thought that the resemblance between the host and parasite was due to shared ancestry rather than mimicry (Chapin 1917; Chapin 1954; Friedmann 1960; Kunkel 1969). However, as the phylogenetic relationships within the group have been resolved through the work of Robert Payne, Michael Sorenson, Nedra Klein and Jeff DaCosta (DaCosta and Sorenson 2016; Klein and Payne 1998; Sorenson and Payne 2001), and the sister relationship between the Viduidae and the Estrildidae confirmed, it has become unquestionable that *Vidua* must have independently converged on their host nestling's appearance.

Figure 1.1. Breeding males of the species of parasitic whydah (left column) and their estrildid hosts (right) at our study site in southern Zambia. Parasite-host pairs: Pin-tailed Whydah (*Vidua macroura*) – Common Waxbill (*Estrilda astrild*) (top), Long-tailed Paradise Whydah (*V. paradisea*) – Melba Finch (*Pytilia melba*) (middle), Broad-tailed Paradise Whydah (*V. obtusa*) – Orange-winged Pytilia (*P. afra*) (bottom). All photos by Gabriel Jamie

Figure 1.2. Breeding males of the species of parasitic indigobird (left column) and their estrildid hosts (right) at our study site in southern Zambia. Parasite-host pairs: Purple Indigobird (*Vidua purpurascens*) – Jameson's Firefinch (*Lagonosticta rhodopareia*) (top), Village Indigobird (*V. chalybeata*) – Red-billed Firefinch (*L. senegala*) (bottom). All photos by Gabriel Jamie.

Initial progress in our understanding of *Vidua* behaviour and parasite-host relationships and song mimicry arose from studies by bird-keepers with little experience of the birds in their natural habitat (Nicolai 1964; Nicolai 1969). However, through the amazing work of Robert Payne who, with Laura Payne, travelled across Africa recording the behaviour of *Vidua* finches, our current understanding of *Vidua* host-use and species limits has been established (Payne 1973; Payne 1985; Payne 1996; Payne 1998). This work has been extended by Michael Sorenson, Chris Balakrishnan, Jeff DaCosta, Justin Schuetz and others carrying out fieldwork and genetic studies to improve our understanding of the radiation (Balakrishnan et al. 2009; Balakrishnan and Sorenson 2007; DaCosta and Sorenson 2014; DaCosta and Sorenson 2016; Lansverk et al. 2015; Mills 2010; Schuetz 2005a; Schuetz 2005b; Sefc et al. 2005; Sorenson et al. 2004; Sorenson et al. 2003).

The most basal member of the Viduidae is the Cuckoo Finch (*Anomalospiza imberbis*) (Sorenson and Payne 2001). It is the only member of the Viduidae not in the genus *Vidua.* While the Cuckoo Finch is also an obligate brood parasite, it has very different breeding strategies to that of the other Viduidae, more closely resembling that of the Common Cuckoo (*Cuculus canorus*). Cuckoo Finch parasitise a range of hosts in the Cisticolidae family (prinias, cisticolas and allies) (Spottiswoode and Stevens 2011). Individual female Cuckoo Finch specialise on a single host species and produce eggs which mimic those of its host (Spottiswoode and Stevens 2010; Spottiswoode and Stevens 2011; Spottiswoode and Stevens 2012). However, at the species level, different female Cuckoo Finches parasitise different hosts, resulting in host-specific races or "gentes" existing within the species. The species that Cuckoo Finches parasitise have varied and complex egg colours and patterns which each host race mimics. By contrast, the nestlings of Cuckoo Finch hosts are unornamented, as is the rule for most passerines, and so are the nestlings of Cuckoo Finches. Therefore, the situation is reversed compared to that found in *Vidua* finches, whose hosts have uniform eggs but remarkably diverse nestlings.

Vidua finches display the hallmarks of an adaptive radiation (Schluter 2000): they are a monophyletic group that have rapidly diversified in the recent past (Gomes et al. 2016; Sorenson et al. 2004), each species occupies on a different ecological niche (host species in the case of *Vidua*) and has evolved specialist adaptations to exploit that niche. These host-specific adaptations include mimetic mouth markings that function to solicit parental care from the host parents (Payne et al. 2001; Schuetz 2005b).

Why is it that some adaptive radiations contain hundreds of species, whereas others contain only tens of species (Schluter 2000)? Two key factors that limit speciation in adaptive radiations are: (i) the potential for colonising new niches ("ecological opportunity") (Schluter 1988; Schluter 2000), and (ii) the extent to which colonising and adapting to those new niches generates reproductive isolation from lineages exploiting other niches. The potential for colonising new niches depends on the availability of unexploited resources in a landscape, coupled with a lineage's suitability to shift from the current resource they are using to the new one. Unexploited resources become available when organisms alter their ranges and colonise new habitats, when there are dramatic shifts in climate, or when the organisms previously exploiting those resources go extinct (Schluter 2000). The extent to which a lineage can exploit that new resource will depend on whether their ancestral niche has equipped them with the necessary pre-adaptations to begin to exploit the new one. Colonisation of new niches results in reproductive isolation if it reduces the frequency of matings between organisms using different niches (pre-mating isolation), or if it reduces the fitness of offspring resulting from such matings (post-mating isolation).

To understand why the *Vidua* radiation has diversified to the extent it has, it is useful to explore how "ecological opportunity" and the link between niche colonisation and speciation apply to this system. For *Vidua* lineages, ecological opportunity represents potential host species overlapping in geographical range, to provide the opportunity for parasitism. Areas that are species rich in potential hosts should also be species-rich in *Vidua*. This is what one finds when looking at the distribution of potential *Vidua* host species in Africa: savannah and grassland habitats in west Africa, which possess the greatest species richness of estrildid finches, also possess the greatest richness of *Vidua* (Schidelko et al. 2011). As the ranges of hosts change in response to changes in climate and vegetation, they become exposed to different species of *Vidua*, which sets up the potential for new host colonisations (Péron et al. 2016).

Figure 1.3 The diverse appearances of estrildid finch nestlings. Top left: Red-billed Firefinch (*Lagonosticta senegala*); top right: Locust Finch (*Paludipasser locustella*); bottom: Melba Finch (*Pytilia melba*). The photo of the Locust Finch is the first photo ever taken of the nestling of this species (details in Jamie 2016). Top two photos by Gabriel Jamie, bottom photo by Claire Spottiswoode.

The link between niche colonisation and speciation is remarkable and unusual in this system. This is because reproductive isolation in *Vidua* is tightly linked to the colonisation of new host species (Sorenson et al. 2003), owing to male and female *Vidua* imprinting on their hosts (Payne et al. 1998; Payne et al. 2000). Male *Vidua* incorporate the vocalisations of their host into their display songs (Payne et al. 1998). Female *Vidua* acquire a mate preference for male *Vidua* who sing like the host she was raised by (Payne et al. 2000). Additionally, female *Vidua* acquire a preference to parasitise the same host species as she was raised by (Payne et al. 2000). The result is that male and female *Vidua* that have been raised by the same host species tend to interbreed (Payne et al. 2000). Due to the imprinting of host preference, these parasite-host associations can be maintained over many generations, allowing the evolution of host-specific adaptations. If a female *Vidua* accidentally lays in the nest of a new host species, she has the potential to initiate a *Vidua* lineage associated with that host (Payne et al. 2002). Male offspring raised in this new environment will grow up to imitate the song of the new host, and female offspring will grow up to be attracted to such males and parasitise the same host. In this way, a new lineage of *Vidua*, reproductively isolated from lineages exploiting different hosts, is established. Therefore, plasticity in mating and host preferences, mediated by early life experience, generates pre-mating isolation between *Vidua* lineages exploiting different hosts. Additionally, the evolution of host-specific mimicry in different lineages generates post-mating isolation. If *Vidua* carrying the genes necessary to exploit different hosts were to interbreed, their offspring would have intermediate mouth markings between the two parental host species, and would likely be inferior at soliciting investment from the host parent compared to a mimetic chick. Therefore, as *Vidua* colonise new hosts they become reproductively isolated from other host lineages, due to both pre- and post-mating isolating factors (Payne and Bonan 2017b).

The speciation process in *Vidua* differs from that of classic radiations. The traditional sequence in adaptive radiation is that divergent selection pressures between populations exploiting different niches generate different ecological adaptations in each lineage. This in turn produces pre-mating and post-mating reproductive isolation as a by-product of these adaptations (Schluter 2000). For example, in Darwin's Finches the speciation process can be characterised as follows (reviewed in Grant and Grant 2008): finch lineages evolve to specialise on different seed types. Over several generations this results in finch lineages specialising on each seed type evolving different beak shapes. Matings between individuals

Figure 1.4. A parasitised clutch of a Common Waxbill. The Pin-tailed Whydah egg is the larger one on the top right. The remaining five eggs belong to the waxbill. Photo by Gabriel Jamie.

Figure 1.5. A newly-hatched Pin-tailed Whydah chick (left) alongside a newly-hatched Common Waxbill chick (right). Note the darker skin, larger size and white wing tufts of the Pin-tailed Whydah. Photo by Gabriel Jamie.

of different beak shapes results in offspring that have intermediate beak shapes that are poorly adapted to consume either resource that their parents are exploiting. This results in post-mating isolation between lineages. Additionally, there is some evidence that male song parameters also vary with beak shape, altering male display (Huber and Podos 2006; Podos 2001). Females have been shown to prefer to mate with males that sing like their fathers, and this further promotes pre-mating isolation between lineages (Grant and Grant 1997). By contrast, in *Vidua,* the sequence is reversed. Pre-mating reproductive isolation arises first, and in only a single generation, because of imprinting on hosts by both sexes (Payne et al. 1998; Payne et al. 2000). Host-specific genetic adaptations, such as mimetic mouth markings, arise later over many generations as the parasite-host relationship is maintained and parasites adapt to their new niche. For this reason, understanding the extent of the radiation rests far more on understanding the factors leading to successful exploitation of new niches, than on explaining how local genetic adaptation can produce reproductive isolation as a by-product.

The tight link between host colonisation and speciation in *Vidua* means that if we can understand what limits host colonisation in this group, we can also understand what limits speciation. We can then explain why the *Vidua* have radiated specifically to 19 species rather than many more or fewer species. This is a more precise question than just asking why a radiation is so species-rich or species-poor. As the processes of speciation, mimicry and parasitism are uniquely linked *Vidua* finches, they provide a very good vertebrate system in which to address these questions.

An important question in our understanding of adaptive radiations is how species shift to exploit novel niches despite only possessing the genetic adaptations necessary to exploit their ancestral niche. Does the process of specialisation to a particular niche restrict the potential of that lineage to subsequently take advantage of new ecological opportunities? In other words, how do specialists colonise new niches? There are two potential solutions. First, the new niche may be similar enough to the ancestral niche that there is some transfer of fitness from the one environment to the other. For example, a study on parasitic feather lice found that lice transferred to avian hosts of a similar body size to their natural host survived better than those transferred to hosts that were much bigger or smaller than the natural host (Bush and Clayton 2006). If hosts show strong phylogenetic signal in traits that are key for parasite survival, we might expect more closely-related hosts to present more similar environments to colonising parasites (Poulin and Keeney 2008; Poulin et al. 2011). This leads to the prediction that switches between more distantly-related host environments should result in lower transfer of fitness for parasites from one to another. This was found in a study on nematode parasites of *Drosophila* flies, which showed that experimental infections of more phylogenetically distant novel hosts were less likely to succeed than those to more closely related ones (Perlman and Jaenike 2003).

Second, the parasite may exhibit phenotypic plasticity in traits that are necessary to adapt to the novel environment. Phenotypic plasticity allows traits to develop differently in different environments (West-Eberhard 2003), allowing organisms to generate an immediate phenotypic response to an environment. Such plasticity could facilitate lineages to persist for long enough in a new environment to acquire the necessary genetic adaptations to prosper there (Levis and Pfennig 2016; Pfennig et al. 2010; Price et al. 2003; West-Eberhard 2003). In other words, plasticity can allow organisms to shift from one adaptive peak to another without having to traverse valleys of low fitness in between (Pfennig et al. 2006; Price et al.

2003). For example, studies on the begging calls on the brood-parasitic Horsfield's Bronze Cuckoo (*Chalcites basalis*) have shown that nestling cuckoos can develop different begging calls in different host environments through interacting with host parents (Langmore et al. 2008). This allows the cuckoo to persist in a range of host environments, by allowing it to produce host-specific begging calls without suffering the costs of specialisation.

Thesis format

In the context of *Vidua* finches, the roles of host relatedness and parasite plasticity in limiting successful colonisation of new hosts can be investigated with a mixture of experimental and comparative approaches. In this thesis, I experimentally transfer *Vidua* to a novel host species and measure survival and plasticity in the new environment (Chapter 5). I also carry out a comparative study of the evolution of host mouth markings to see whether there is strong phylogenetic signal in this key trait, and what ecological factors might have influenced its evolution (Chapter 6). Prior to these chapters, two conceptual and review chapters provide a framework in which to situate the mimetic adaptations shown by *Vidua*.

First, in Chapter 2, I critically examine the logic by which mimicry in the natural world can be conceptually organized and analysed. I highlight key criteria with which to differentiate mimicry in nature, focussing on the information content, reliability and intended receivers of the mimic's signal. This framework can be applied to compare the different forms of host mimicry exhibited by *Vidua.* For example, *Vidua* are known to mimic the appearance of host nestlings as well as the songs of host adults. How are these two forms of mimicry related to one another? Are they the same type, or are there fundamental differences? This chapter has been published in *Proceedings of the Royal Society B* (Jamie 2017).

Before investigating whether *Vidua* show mimicry of hosts in traits other than mouth markings and whether they show plasticity, it is necessary to have clear predictions. In Chapter 3, I review the literature on begging call mimicry and development across all avian brood parasite species. As part of this review, I outline the conditions under which we expect begging call mimicry to evolve, and when we expect it to develop primarily through genetic or environmental cues. This provides clear predictions for what we expect to occur in *Vidua* finches, which are later tested in Chapters 4 and 5. Chapter 3 has been accepted to form a

book chapter co-authored with Rebecca Kilner in an upcoming book on avian brood parasitism edited by Manuel Soler.

In Chapter 4, I provide the first quantitative evidence that *Vidua* nestlings mimic the begging calls as well as the mouth markings of their specific host species. I also show for the first time that *Vidua* are imperfect mimics of their hosts, and demonstrate consistent differences in both mouth markings and begging calls between parasites and hosts. I evaluate the relative merits of different hypotheses to explain these imperfections. Overall, this chapter provides the first quantitative evidence of host-specific adaptations by *Vidua*.

In Chapter 5, I simulate the colonisation of a new host by transferring *Vidua* eggs into the nest of a new host species. I monitor *Vidua* survival in the foreign host environment and test several hypotheses about what explains differences in chick survival. I test whether *Vidua* can plastically shift their begging calls in the new environment to improve survival. Using DNA metabarcoding, I investigate whether there are dietary differences between host species which could account for differences in survival.

In Chapter 6, I carry out a comparative analysis on the evolution of estrildid mouth markings. Estrildid finches are the hosts of *Vidua* and so provide the "landscape" of ecological niches that *Vidua* may colonise and adapt to. I test whether the host family shows strong phylogenetic signal in mouth marking traits, and investigate what ecological forces may have shaped the evolution of estrildid mouth markings. I also examine whether parasitism by *Vidua* has altered the mouth marking evolution of the hosts.

Field work

Chapters 4, 5 and 6 incorporate data collected through field work carried in the Choma District of southern Zambia in January–April 2014, 2015, 2016 and 2017 (Figure 1.6). Data were collected principally from within an area of about 40 km^2 around Musumanene (16°47′S, 26°54'E) and Semahwa Farms. The area is a mosaic of deciduous broad-leaved (miombo) woodland, grassland and agricultural fields. The primary crop being farmed is tobacco (*Nicotonia tabaccum*, which is an intensive crop, such that relatively small areas of land are cleared for growing tobacco while other fields are left fallow. This produces a mixture of secondary growth and edge habitat that abuts the miombo woodland, in which

many estrildid species are common. The field site is located on the Choma plateau with an elevation varying between around 1300 m and 1390 m above sea level; in fact, the highest point in southern Zambia is located on the field site. There is a single rainy season in the area, lasting from late November to early April. This is followed by a dry winter season from May to August, and a hot, dry season from September to October. Estrildid finches and their *Vidua* hosts breed in the area during the rainy season, with a peak in most species around February and March when seeding grasses are abundant.

Several species of estrildid finch and their *Vidua* parasites occur in the Choma area. Among the whydahs, Pin-tailed Whydah are common in open and wet habitats along with its host Common Waxbill. Slightly less common are Long-tailed Paradise Whydah (*V. paradisea*), Broad-tailed Paradise Whdyah (*V. obtusa*) and their respective hosts, Melba Finch (*Pytilia melba*) and Orange-winged Pytilia (*P. afra*). Two indigobird species occur commonly, Purple Indigobird (*V. purpurascens*) and Village Indigobird (*V. chalybeata*) along with their respective hosts Jameson's Firefinch (*Lagonosticta rhodopareia*) and Red-billed Firefinch (*L. senegala*). Zambezi Indigobird (*Vidua codringtoni*) has been reported from the area in the past (P. M. Leonard *pers. comm.*) but was not found during 2014–2017 despite repeated searches. Its host, Red-throated Twinspot (*Hypargos niveoguttatus*), occurs frequently in thicket vegetation. We were unable to find any nests of this species during 2013–2017, and so this is the only regularly occurring estrildid species for which no mouth marking photos or begging call recordings were obtained. Additionally, there are several estrildid species breeding at the field site which are not regular hosts to *Vidua* finches. These are Blue Waxbill (*Uraeginthus angolensis*), Bronze Mannikin (*Spermestes cucullatus*), African Quailfinch (*Ortygospiza fuscocrisa*), and Zebra Waxbill (*Amandava subflava*). Locust Finch (*Paludipasser locustella*) breeds in seasonally flooded grasslands and was recorded in the area only in the 2015 and 2016 field seasons. We found three Locust Finch nests during the 2016 season and were able to photograph the chicks (Figure 1.3, top right). These are the first ever photos of the nestling of this scarce species (Jamie 2016).

Predation of estrildid nests was a recurrent issue, partially mitigated by hatching eggs in an incubator rather than in the field. Trail camera were put up outside nests in a non-systematic way to get a sense as to what the main predators were. Boomslangs (*Dispholidus typus*) were recorded on video predating Common Waxbill nests on three occasions. An African Grey Hornbill (*Tockus nasutus*) was once video-recorded predating a Blue Waxbill

nest. Other possible predators were Vervet Monkeys (*Chlorocebus pygerythrus*) and humans (*Homo sapiens*).

Estrildid finch nests were found by people working and living on the farms. At the start of each field season, I gave a presentation on the species we were looking for. Printed sheets were given to people in the area with images of the birds we were after, as well as how much we would pay if an active nest was shown to us. Once nests were shown, their location was recorded on a GPS. The contents and developmental stage (estimated by "candling" with a strong torch) of each nest were recorded in a notebook. Nests were re-visited on subsequent days to collect more data and to carry out the experiments reported in Chapters 4, 5 and 6. Eggs were taken back to an incubator set up in the farmhouse we were staying in to be used in transfer experiments and for ultra-violet (UV) photography.

Figure 1.6. Field work on *Vidua* finches in Choma, southern Zambia. Top left: with some of the nest-finders at Semahwa Farm (photo by Amos Richards); top right: with (left to right) Junior, Lazaro Hamusikili, Collins Moya and Tom Hamusikili about to set out by boat to find Zebra Waxbill nests in the reeds (photo by Gabriel Jamie). Bottom left: recording the begging calls of a Pin-tailed Whydah nestling (photo by Claire Spottiswoode); bottom right: the red Toyota Hilux in which most of the field work in 2014 to 2017 was done (photo by Gabriel Jamie).

Chapter 2:
Signals, cues and the nature of mimicry

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2.1 INTRODUCTION

"Mimicry" is used in the evolutionary and ecological literature to describe diverse phenomena (Table 2.1). These impressive outcomes of natural selection are widely fêted in textbooks and documentaries. Despite their canonical status, there remains considerable lack of clarity over how these resemblances are related to one another and the extent to which they are products of the same evolutionary processes (reviewed in Dalziell and Welbergen 2016; Grim 2005; Grim 2013). Just as recent papers have brought clarity to social evolution by defining and systematising terms used to describe social interactions (Ghoul et al. 2014; West et al. 2007), this review aims to facilitate research on mimicry by proposing a conceptual framework that contrasts and orders mimetic resemblances across sensory modalities and taxonomic groups. Rather than aiming to provide an entirely new classification scheme, this review instead critically examines the criteria by which mimicry is currently differentiated, and then explores them to their logical conclusions.

First, this review highlights an important, but largely overlooked, distinction between "signal mimicry" and "cue mimicry", and suggests an evolutionary pathway for the one form to transition to the other. Second, it examines the criteria which generate the three traditionally recognised mimicry forms (aggressive, Batesian and Müllerian). In uncovering the two key criteria that distinguish these forms, the review highlights the existence of a fourth, largely overlooked form which is a logical extension of the criteria used to delineate the other three. In so doing, the review clarifies the conceptual relationships between these traditional forms of mimicry and shows that our current framework is incomplete unless this fourth form is included (Figure 2.1).

The review's aim is to uncover the criteria by which examples of mimicry are conceptually organised, and not to re-define mimicry. Modern definitions of mimicry are based on the one proposed by Vane-Wright in 1980 (Vane-Wright 1980), and which is widely used in the literature today (e.g. Calhim et al. 2014; Rubio et al.

2013; Welbergen and Davies 2011). Modified versions of Vane-Wright's definition have been suggested, with recent papers on avian vocal mimicry (Dalziell et al. 2015) and mimicry more generally (Dalziell and Welbergen 2016) suggesting that mimicry evolves if:

"a receiver perceives the similarity between a mimic and a model and as a result changes its behaviour in a manner that provides a selective advantage to the mimic."

This definition relaxes the condition of Vane-Wright's 1980 definition that fitness benefits to the mimic must arise from the receiver identifying the mimic as an example of a model. Instead, fitness benefits need only result from the receiver perceiving the similarity between mimic and model. This definition is adopted here and is consistent with the framework outlined in this review article for understanding the relationships between mimetic resemblances.

2.2. SIGNAL VERSUS CUE MIMICRY

(a) Signals and cues

In studies of animal communication, a fundamental distinction is made between signals, which have evolved specifically to alter a receiver's behaviour, and cues, which are incidental sources of information detected by unintended receivers (Maynard Smith and Harper 2003). An example of a signal is the warning colourations of many distasteful insects (Poulton 1890; Ruxton et al. 2004). Selection by predators has led to the evolution of bright, conspicuous and memorable markings that convey information about the prey's toxicity to the predator (Mappes et al. 2005). By contrast, the rustling sound produced by a mouse as it runs through the undergrowth is a cue. A predatory owl uses this sound to gain information about the mouse's location but the trait has not evolved under selection to signal location to predators.

Mimics can simulate both signals ("signal mimicry") and cues ("cue mimicry") of models to alter receiver behaviour. However, this distinction is often overlooked. It is briefly noted in Maynard Smith & Harper (ref. Maynard Smith and Harper 2003, p. 86) but not explored further. An example of signal mimicry is the

similarity in begging calls between some nestling brood-parasitic birds and their hosts. Horsfield's bronze-cuckoos (*Chalcites basalis*) are brood-parasitic birds that lay their eggs in the nests of a variety of other species. The young cuckoo develops begging calls that closely match those of the nestlings of the host species it is being raised by (Langmore et al. 2008). The trait being copied is the model's begging call, which has evolved specifically under selection to transfer information to the host parent.

An example of cue mimicry comes from spiders. A predatory jumping spider (*Portia fimbriata*) attracts orb-web spiders (*Zygiella x-notata* and *Zosis geniculatus*) by vibrating the latter's web to resemble a fly struggling (Tarsitano et al. 2000). The jumping spider is the mimic, the fly is the model, and the orb-web spider is the receiver. The model's traits being copied (the web vibrations of a struggling fly) are cues, as they have not evolved under selection to signal information to the orb-web spider.

To summarise, in signal mimicry, the mimic's signal comes to resemble the model's signals, whereas in cue mimicry, the mimic's signal comes to resemble the model's cues (for examples see Table 2.1). Importantly, in both cue mimicry and signal mimicry, the trait of the mimic is a *signal* to its intended receiver, as it has evolved specifically to alter that receiver's behaviour.

(b) The importance of distinguishing signal and cue mimicry

A key difference between signal mimicry and cue mimicry is that mimic and model share the same intended receiver in signal mimicry, but do not in cue mimicry. This is evolutionarily relevant because the receiver is not just the passive recipient of a signal, but the agent of selection whose perception of and response to that signal determines its adaptive value. Therefore, in signal mimicry, mimic and model have the same agent of selection, whereas in cue mimicry, mimic and model do not.

The distinction between shared and unshared receivers is important, first, because different receivers may vary in their sensory systems and cognition (Dalziell and Welbergen 2016). Therefore, different receivers select for the mimic to simulate

the model's trait in different ways. When one organism converges on the traits of another organism/object through mimicry, it does not perfectly simulate all aspects of the model's trait. Rather, it mimics those aspects of the trait necessary to make the mimic's receiver perceive the similarity between mimic and model (Dalziell and Welbergen 2016). This is a useful attribute of mimicry as it highlights the aspects of the model's traits the mimic's receiver uses to identify the model. Therefore, in cue mimicry, mimic and model's traits may closely resemble one another from the perspective of the mimic's intended receiver, but seem quite different from the perspective of the model's intended receiver. The extent of this discrepancy will depend on the divergence in selection pressures exerted by mimic and model's receivers.

A second reason to distinguish signal and cue mimicry is that when mimic and model share the same intended receiver (signal mimicry), the reliability of the mimic's signal can serve to reinforce or undermine the reliability of the model's. If the mimic's signal is non-deceptive, the mimic's signal will reinforce the reliability of the model's signal to their shared receiver. If the mimic's signal is deceptive, it will undermine the reliability of the model's traits. The more deceptive signal mimics in the population, the less reliable, on average, the signal is to the receiver (Lindström et al. 1997). This may lead to selection for the receiver to no longer avoid/approach the model's phenotype, with detrimental consequences for both model and mimic (Joron and Mallet 1998). By contrast, if mimic and model have different receivers (cue mimicry), the reliability of the mimic's signal should have no impact on the perceived reliability of the model's trait to the model's receiver. An exception would be if the model's intended receiver were also an unintended receiver of the mimic's signal; for example, superb lyrebirds mimic other bird species' calls (Dalziell and Magrath 2012), presumably to attract mates and defend territory, but their vocalisations might also be heard by individuals of the species it is mimicking. This might lead to selection for members of the mimicked species not to respond to the call, as it would often be unreliable.

The distinction between signal and cue mimicry can be illustrated with empirical examples (Table 2.1). In the Horsfield's bronze-cuckoo begging call example (Langmore et al. 2008), the nestling cuckoo (mimic) and host (model) have the same

intended receiver (the host parent). Therefore, the model's trait being copied are signals to the mimic's intended receiver, making this signal mimicry. By contrast, anglerfish draw in prey using a fleshy extension on their head as a lure that resembles the shape and movement of a worm or fish (Pietsch and Grobecker 1978). The intended receiver of the model's traits being mimicked differs from the mimic's intended receiver, making them cues not signals to the mimic's intended receiver.

Importantly, traits are not inherently "cues" or "signals" but can only be classified as such with respect to a given receiver. For example, a male Túngara frog's (*Physalaemus pustolosus*) vocalizations are signals to attract females, but also cues the predatory fringe-lipped bat (*Trachops cirrhosis*) uses to locate frog prey (Halfwerk et al. 2014; Ryan 1985). In the context of mimicry, the receiver with respect to which the model's trait is judged as signal or cue is the intended receiver of the mimic's signal. Therefore, if mimic and model share the same intended receiver, then the model's trait is viewed as a signal and the system can be classed as signal mimicry. If, however, mimic and model differ in their intended receiver (or the model's trait is not a signal in any context), the model's trait is a cue and the system can be classed as cue mimicry. This emphasises the importance of identifying the receivers driving the evolution of both mimic's and model's traits.

(c) Evolving signal from cue mimicry

Cue mimicry and signal mimicry do not necessarily have disparate evolutionary trajectories. Instead, cue mimicry can transition to signal mimicry when the mimic's presence has a fitness consequence on the model; either directly or via the mimic's effects on the receiver. These fitness consequences mean that, once the mimic has reached a sufficient frequency in the environment, there will be selection for the model to alter the trait that is being mimicked either towards or away from that of the mimic. Once this has occurred, the model is now signaling information to the mimic's receiver (i.e. it has become a *signal* to the mimic's receiver) and the system transitions from cue to signal mimicry. So, becoming the model in a cue mimicry system can set the stage for an evolutionary shift in a trait from being a cue to a signal. Interestingly, if the response of the model is to converge on the mimic's signal, the model itself will become a mimic.

Table 2.1. Examples of signal and cue mimicry from the literature. Note how signal and cue mimicry can each be either aggressive, Batesian, Müllerian or rewarding.

	Signal mimicry	**Cue mimicry**
Aggressive	Begging call mimicry by avian brood parasites (Davies et al. 1998; Langmore et al. 2008, Chapter 4) Mimicry of egg signatures by avian brood parasites (Brooke and Davies 1988; Spottiswoode and Stevens 2012) Hydrocarbon mimicry by socially parasitic insects (Kilner and Langmore 2011) Mimicry of a flower by praying mantis species to attract insect prey (O'Hanlon et al. 2014) Mimicry by unrewarding plant a rewarding species to attract pollinators (Newman et al. 2012)	Predatory insects luring spiders by mimicking vibrations of struggling insects (Tarsitano et al. 2000; Wignall and Taylor 2011) Olfactory mimicry of carrion by flowers to attract insects (Johnson 2016) Anglerfish attracting smaller fish using a lure that resembles a prey item (Pietsch and Grobecker 1978) Mimicry of dung by plant seeds to attract dung beetle dispersers (Midgley et al. 2015)
Batesian	Mimicry of aposematic organisms by undefended organisms to avoid predation (Penney et al. 2012) Auditory mimicry of rattlesnake rattles by burrowing owls to avoid predation (Rowe et al. 1986)	Mimicry of bird droppings by certain caterpillar species to avoid predation (Cott 1940) Mimicry of inanimate objects such as stones and dead sticks (Skelhorn et al. 2010b)
Müllerian	Mimicry of aposematic organisms by other defended organisms to avoid predation (Bates 1862; Müller 1879)	Fork-tailed drongo reliably mimicking the alarm calls of another species to signify the presence of a predator (Flower et al. 2014)
Rewarding	Visual mimicry by a plant of other rewarding plant species to better attract pollinators (Benitez-Vieyra et al. 2007)	Mimicry of host song during display by adult male *Vidua* finches (Payne et al. 1998)

To illustrate the transition from cue to signal mimicry I will use mimicry of host eggs by brood-parasitic birds as an example (e.g. Brooke and Davies 1988; Spottiswoode and Stevens 2010). Ancestrally, prior to being parasitized, the colour and pattern of host eggs may have evolved for camouflage or thermoregulation (Kilner 2006a; Stoddard et al. 2011). In copying these host egg features, parasites were initially mimicking the model's cues (cue mimicry). Subsequently, a co-evolutionary arms race ensued in which hosts responded by altering their eggs' appearance to signal information about maternal identity, so hosts can better distinguish their own eggs from parasite eggs (Spottiswoode and Stevens 2010; Spottiswoode and Stevens 2011; Spottiswoode and Stevens 2012; Stoddard et al. 2014). Parasites have tracked this change, altering their own eggs' appearance to deceptively signal the same information as the host's eggs (Spottiswoode and Stevens 2011; Spottiswoode and Stevens 2012). Following the framework proposed here, the phenomenon has shifted from cue mimicry to signal mimicry. Thus, cue mimicry can be the first step on a co-evolutionary path to signal mimicry.

When the model is inanimate, cue mimicry is stable and will not transition to signal mimicry via a co-evolutionary arms race. For example, if the model is a rock (whose appearance is apparently copied by stone plants, *Lithops* (Barrett 1987)), it cannot evolve to alter its appearance.

(e) "Masquerade" as a special case of cue mimicry

How does "masquerade" fit within the framework proposed here? The modern definition of masquerade stems from Endler (1981), who considered masquerade as the adaptive resemblance of an organism to an inanimate or inedible object (Endler 1981). This definition of masquerade was updated by Skelhorn et al. (2010) (Skelhorn et al. 2010a). They considered Endler's 1981 definition to exclude certain resemblances that might intuitively be considered masquerade and suggested the following formal definition:

"one whose appearance causes its predators or prey to misclassify it as a specific object found in the environment, causing the observer to change its behaviour in a way that enhances the survival of the masquerader. Any change in the population/

evolutionary dynamics of the model caused by the presence of the masquerader will not be as a result of the signal receiver changing its behaviour towards the model" (Skelhorn et al. 2010a)

In many publications since, the second part of the definition of masquerade – that the masquerader must not influence the population/evolutionary dynamics of the model by changing the receiver's behaviour – is left out. Instead the focus is on the "inanimate", "inedible" or "uninteresting" nature of the model (e.g. Skelhorn et al. 2010b; Skelhorn and Ruxton 2010; Stoddard 2012). It has also been formulated as situations in which the model is "ignored" by the receiver (Dalziell and Welbergen 2016).

The "inanimate" and "uninteresting" aspects to models in masquerade systems place them in the category of cue mimicry. If the models being copied are inanimate, their traits cannot evolve to become signals and, if they are uninteresting, they have not evolved to signal information to a receiver. From the perspective of shared (signal mimicry) versus unshared (cue mimicry) receivers, masquerade also falls within cue mimicry. If the model is "uninteresting" to the mimic's intended receiver, then the model must have a different intended receiver from the mimic (or have no intended receiver at all).

To summarise, masquerade can be considered a special case of cue mimicry in which the model is inanimate, uninteresting and inedible.

2.3. SUB-DIVIDING SIGNAL AND CUE MIMICRY: AGGRESSIVE, BATESIAN, MÜLLERIAN AND REWARDING MIMICRY

Section 2 emphasised the importance of the signal versus cue mimicry distinction. This section now revisits the traditional subdivisions within mimicry. It aims to find clear and evolutionarily relevant criteria that separate mimicry types from one another and take them to their logical conclusions (Figure 2.1). Clear criteria not only help us to draw comparisons between seemingly disparate examples of mimicry but also highlight how the different forms of mimicry can evolve from one type to another.

Efforts to sub-divide mimicry were first formalised with Vane-Wright (Vane-Wright 1976), who separated mimicry types according to the interactions between the three parties: receiver, mimic and model. These depended on three distinctions: 1) whether the mimic's presence had a positive effect on the model's fitness or a negative one. 2) Whether the receiver's "biological roles" with respect to the model and to the mimic are "aggressive" or "protective" and 3) whether the model, mimic and receiver are all the same species, all different species or only two of the same species. The various permutations of these criteria result in him identifying forty different types of mimicry (Vane-Wright 1976).

Here, I suggest a conceptual organisation of mimicry based on the information content of the mimic's signal to the receiver. This framework accommodates the three general types of mimicry commonly recognized today (aggressive, Batesian, Müllerian), and highlights a fourth, often overlooked, form for which I suggest the term "rewarding mimicry" Here mimicry is organised according to two axes, information content and deceptiveness.

First, information content: does the mimic signal a fitness cost (punishment) or benefit (reward) to manipulate receiver behaviour? Organisms can manipulate receiver behaviour by either promising a reward or a punishment. For example, an inedible butterfly species uses aposematic colouration to signal its distastefulness to receivers and avoid being eaten. By contrast, a nectar-containing flower signals its rewarding nature through a conspicuous flower to encourage pollinators to visit it. Similarly, in copying the traits of models, mimics manipulate receiver behaviour by presenting the potential of a reward or punishment.

Second, deceptiveness: is the mimic's signal deceptive? In some situations, the perceived punishment is "real", such as when multiple distasteful butterfly species evolve to resemble one another. In others, it is "false", such as when an edible butterfly species has evolved to resemble an inedible one. The degree of discrepancy between the mimic's advertised reward/punishment and the actual levels of reward/punishment is a measure of how deceptive the mimic's signal is.

The framework presented here is not hierarchical and the two criteria can be applied in either order with neither having priority over the other. The framework can

be visualised as a 2-dimensional graph divided into four quadrants (Figure 2.1). The four quadrants on the graph do not signify discrete categories of mimicry. Instead they help to define two important axes along which examples of mimicry vary. It is useful to think of them as the four points on a compass that can help us to position mimicry systems relative to one another across a "mimicry landscape". Clear criteria defining these extremes facilitate comparisons between examples of mimicry, and clarify the mechanisms through which they can transition from one type to another.

1) Aggressive mimicry

In aggressive mimicry, the mimic signals a fitness benefit to the receiver and the mimic's signal is deceptive. More generally, a system can be classified as aggressive mimicry when the advertised benefits to the receiver are lower than the actual benefits.

An example of aggressive signal mimicry is a praying mantis that has evolved to resemble a flower to attract insect prey (O'Hanlon et al. 2014). The mantis deceptively signals a fitness benefit to the receiver, exploiting the flower's attractive signals to the pollinator to gain access to prey. Other examples include, *Bolas* spiders mimicking the sexual attractant pheromones of female moths to attract male moths as prey items (Eberhard 1977) or sexually-deceptive plants attracting male pollinators (Ellis and Johnson 2010; Jersakova et al. 2006).

The anglerfish system referred to earlier (Pietsch and Grobecker 1978) is an example of aggressive cue mimicry. Anglerfish draw in prey using a fleshy extension on their head as a lure. This is an example of cue rather than signal mimicry, because the model's traits being copied are cues (not signals) to the anglerfish's intended receiver. It is aggressive mimicry because the signal produced by the anglerfish is unreliable and the receiver would gain a fitness benefit from interacting with the model (it would eat a food item) whereas it would suffer a fitness cost from interacting with the mimic (it gets eaten).

2) Batesian mimicry

In Batesian mimicry, the mimic signals a fitness cost to the receiver and the mimic's signal is deceptive. More generally, a mimicry system can be classified as Batesian mimicry when the advertised costs to the receiver are greater than the actual costs.

Examples of Batesian signal mimics include *Papillio* swallowtail butterflies resembling defended butterfly species (Kunte 2009) and harmless hoverfly species (family Syrphidae) resembling defended wasps and bees (order Hymenoptera) (Penney et al. 2012; Rotheray and Gilbert 2011).

By contrast, an example of "Batesian cue mimicry" would be an undefended caterpillar that resembles a bird dropping. This is cue rather than signal mimicry because the traits of the model (a bird dropping) being copied have not (as far as is known) evolved to signal information to the caterpillar's intended receiver (probably an avian predator). It is classified as Batesian because the model is deceptively signalling a fitness cost to the receiver.

3) Müllerian mimicry

In Müllerian mimicry, the mimic signals a fitness cost to the receiver and the mimic's signal is non-deceptive.

An example of Müllerian signal mimicry comes from *Heliconius* butterflies in which multiple toxic species converge on the same phenotype under selection to signal their distastefulness to predators (Bates 1862; Mallet and Joron 1999; Turner 1981). The mimic non-deceptively signals a fitness cost to the receiver to manipulate its behaviour, making it Müllerian. It is signal mimicry because mimic and model share an intended receiver (avian predators) of the mimicked trait (wing patterns). There are numerous other examples of Müllerian signal mimicry such as in catfish (Alexandrou et al. 2011), birds (Dumbacher and Fleischer 2001) and velvet ants (Mutilidae) (Wilson et al. 2015).

A possible example of Müllerian cue mimicry would be a distasteful organism that resembles a distasteful inanimate object under selection to more effectively signal

its distastefulness to predators. For example, if a caterpillar that looked like a bird dropping was itself distasteful, then this would be an example of Müllerian cue mimicry. It would be interesting to review known resemblances of organisms to animal droppings and see whether, in any of these cases, the mimic is itself unpalatable to its intended receiver.

Examples of both Müllerian cue and Müllerian signal mimicry come from fork-tailed drongos (*Dicrurus adsimilis*) depending on who the intended receiver of the drongo's call is. Drongos produce a variety of alarm calls while foraging alongside other species. Sometimes these alarm calls are produced when a predator is present ('true' alarm calls), and sometimes they are produced when there is no predator ('false' alarm calls). The calls can either be drongo-specific, or mimic calls of a range of other species it forages alongside. By using a mix of honest and deceptive alarm calls, drongos cause heterospecific foragers to drop their prey in response to the perceived risk of attack by predators and the drongo is then able to seize the deserted prey (Flower 2011; Flower et al. 2014). When the drongo uses mimicry to direct alarm calls at other foraging birds when a predator is really present, the signal is reliable and the drongo is alerting the receiver to a real danger, but uses the 'voice' of another species to do so. This constitutes Müllerian signal mimicry in the case where model and receiver are the same species (mimic and model share a receiver), and Müllerian cue mimicry when model and receiver are different species (mimic and model have different intended receivers of their calls).

4) "Rewarding" mimicry

The fourth permutation, in which the mimic signals a fitness benefit to the receiver and the mimic's signal is non-deceptive, is one that is rarely identified and for which the term "rewarding mimicry" is proposed here. Whilst the possibility of Müllerian-like systems based on profitability rather than unprofitability has previously been acknowledged (Benitez-Vieyra et al. 2007; Johnson and Schiestl 2016; Sherratt 2008), it has always been classified within Müllerian mimicry.

Plant-pollinator interactions could provide the best systems in which to look for examples of rewarding signal mimicry. Here, multiple species of plants may gain

a benefit by using the same flower phenotype to signal to shared pollinators. The mimic's signal is reliable to the receiver (the pollinator) as both mimic and model plants reward the pollinator with nectar. Such interactions have been noted by other authors but they have generally classified them under Müllerian mimicry (Benitez-Vieyra et al. 2007; Johnson and Schiestl 2016; Sherratt 2008) or more broadly under "non-deceptive" mimicry (Dalziell et al. 2015). An example of reliable mimicry in plant-pollinator interactions has been identified between plants of the families Turneraceae and Malvaceae (Benitez-Vieyra et al. 2007). A rewarding species of Turneraceae (*Turnera sidoides*) was shown to resemble co-flowering species of Malvaceae and to gain higher pollination levels when growing together with the model plant than when growing alone (Benitez-Vieyra et al. 2007). This example is classified as Müllerian mimicry by the paper's authors; however, given that the mimic signals rewards rather than punishment to manipulate receiver behaviour, this is better classed as rewarding mimicry. This is rewarding *signal* mimicry because mimic and model share the same intended receivers (pollinating insects).

An example of rewarding cue mimicry comes from the brood-parasitic *Vidua* finches of Africa. Both male and female *Vidua* imprint on their host species with males growing up to mimic the songs of their host and females acquiring a mate preference for males who sing like the host she was raised by (Payne et al. 2000; Payne et al. 2001). Here, the mimic is the adult male *Vidua*, the model is an adult of its host species and the receiver is the adult female *Vidua*. Male *Vidua* use mimicry to reliably signal to female *Vidua* information about their early natal environment (in which species' nest he was raised). Females perceive the similarity between the male *Vidua*'s song and that of their own host and alter their behaviour accordingly. The model's trait being mimicked (its song) has not evolved under selection from female *Vidua;* instead, the intended receiver of the hosts song is other members of its own species. For these reasons, this example shows characteristics of rewarding cue mimicry.

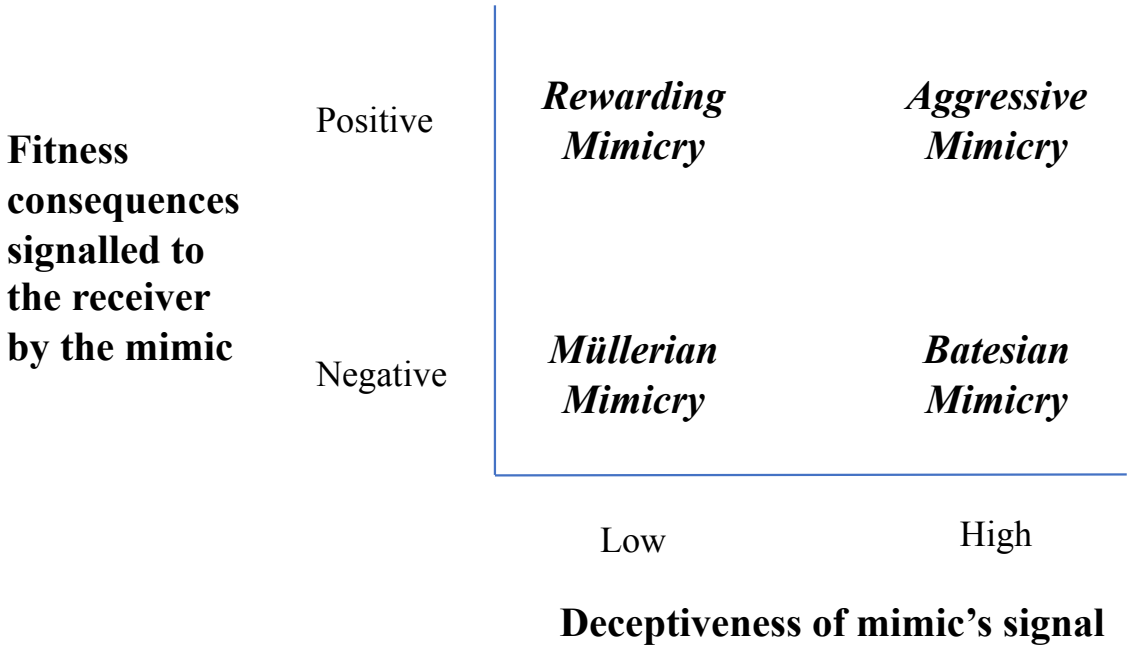

Figure 2.1. How mimetic resemblances can be categorized based on the deceptiveness of the mimic's signal and the fitness consequences signalled by the mimic in order to manipulate receiver behaviour.

2.4 SIGNAL DECEPTIVENESS AND TRANSITIONS BETWEEN MIMICRY TYPES

Shifts in the levels of deceptiveness shown by mimics can result in transitions from one mimicry type to another. Mimics vary in the degree to which their signals are deceptive (Vane-Wright 1976). The mimic's signal is deceptive in Batesian and aggressive mimicry, but not so in Müllerian and rewarding mimicry.

Both Müllerian and rewarding mimicry are susceptible to cheating. In rewarding mimicry, cheats may reduce investment in the reward for the receiver, making their signal deceptive. In so doing the system would transition from rewarding towards aggressive mimicry. Aggressive mimicry is found in non-rewarding plants that look like a rewarding species, thus duping pollinators to visit them (Johnson and Schiestl 2016; Schaefer and Ruxton 2009). Whilst some authors classify this

resemblance as Batesian mimicry (Schaefer and Ruxton 2009), it is classified as aggressive mimicry here because the mimic is signalling a reward to the receiver. By contrast, if a Müllerian mimic were to cheat by reducing investment in toxicity, the system would transition to Batesian mimicry (Figure 2.1).

If co-mimics differ from one another in their degree of toxicity the system is sometimes termed "quasi-Batesian" mimicry as the mimic is not entirely undefended, just less so than the model (Speed 1999). Similarly, if rewarding mimics were to cheat by decreasing investment in rewards relative to co-mimics, this system could be classified as "quasi-aggressive" mimicry.

2.5. POSITIONING DIFFICULT EXAMPLES IN THE FRAMEWORK: MASQUERADE AND AVIAN VOCAL MIMICRY

Finally, I consider some cases that may seem difficult to position within the mimicry framework outlined in figure 2.1, focussing on masquerade systems and avian vocal mimicry.

In masquerade, it can be difficult to know whether mimic is signalling a fitness cost or benefit to the receiver. For example, consider a praying mantis that resemble dead leaves to allow the mantis closer access to prey before striking. Here, the model is the dead leaf, the mimic is the mantis and the receiver is the insect prey. There are two ways to look at this situation. One is to take the absolute levels of reward/punishment being advertised by the mimic's signal, which, in this situation, is effectively neutral. When these examples are plotted on Figure 2.1, they fall at the border between Batesian and aggressive mimicry as the mimic's signal is deceptive, but the mimic is signalling neither fitness benefits nor punishment to the receiver. If the relationship between the receiver and the model changes for any reason, such that the receiver now has reason to avoid or engage with the model, the mimicry system would transition from masquerade to Batesian or aggressive mimicry.

A second way to classify these masquerade examples would be to compare the advertised fitness benefits/costs to the receiver of the mimic's signal with the actual fitness costs/benefits. In aggressive mimicry, the advertised fitness costs to the

receiver are less than the actual costs, whereas in Batesian mimicry the advertised fitness costs are greater than the actual costs. In the case of a praying mantis that has evolved to resemble a dead leaf, under selection to allow closer access to prey items, it can be considered aggressive mimicry as the advertised costs (an inconsequential, non-predatory dead leaf) are lower than the actual costs to the receiver (it gets eaten). However, if selection to resemble a dead leaf has been driven by selection from predators, then the system can be thought of as Batesian mimicry as the advertised costs to the receiver (wasted time and energy trying to eat a dead leaf) are greater than the actual costs (getting a meal). Whilst this might seem an arbitrary distinction given that the resulting mimetic phenotype of the mantis is similar in both scenarios, it has been generated by different selection pressures (different receivers) and employed in different contexts. This latter way of classifying masquerade systems is preferable as it is an extension of the logic which classifies a less toxic butterfly species mimicking a more toxic species as a (quasi-)Batesian mimic (Speed 1999), or a less rewarding plant species mimicking a more rewarding one as a (quasi-)aggressive mimic.

Some instances of avian vocal imitations where birds imitate the vocalisations of other species in mate attraction and/or territory defence may also seem difficult to situate in the framework. Returning to the definition of mimicry stated in the introduction, avian vocal imitation is only considered mimicry if "*the receiver receiver perceives the similarity between a mimic and a model and as a result changes its behaviour in a manner that provides a selective advantage to the mimic."* (Dalziell and Welbergen 2016; Dalziell et al. 2015). According to this, only those instances of avian vocal mimicry in which the receiver perceives the similarity between mimic and model vocalisations can be considered mimicry. In many instances of vocal imitation for mate attraction the bird may just be using the sounds in the surrounding environment to help direct the development of its own call (Dalziell et al. 2015; Howard 1974; Kelley et al. 2008). The receiver is not necessarily perceiving the resemblance of the call to a locally occurring species. Receivers may instead just be selecting for large vocal repertoires rather than mimicry *per se*, with imitation of other species just providing a fruitful source of new vocal material for displaying birds. This hypothesis makes the testable prediction that there would be no different fitness outcomes if the repertoire of a mimetic species was expanded using non-local or local species. For example, male Marsh Warblers (*Acrocephalus*

palustris) imitate African bird species whilst vocalizing on their European breeding grounds (Dowsett-Lemaire 1979). Female Marsh Warblers are unlikely to be perceiving similarity between the male's calls and that of the African species given that the female may have wintered in a different area to the male and not encountered those African species. If this is true, then these examples fall outside the definition of mimicry.

By contrast, in those examples of avian vocal imitation where the receiver alters its behaviour due to perceiving a similarity between mimic and model vocalisations the framework can be applied as described in several examples throughout this review to position these systems within the mimicry landscape (e.g. drongo alarm call mimicry, *Vidua* host mimicry, cuckoo begging call mimicry). Again, the information content of the mimic's signal can be used to position it in the framework by considering whether it is deceptive, and the extent to which it advertises rewards or punishment to manipulate receiver behaviour.

2.6. CONCLUSIONS

The conceptual framework presented in this paper provides a set of criteria to categorize and compare examples of mimicry across sensory modalities. The close focus on definitions is not just semantic, but instead draws attention to the commonalities and differences in the processes underlying the evolution of mimicry. It is hoped it will act as a guide with which to conceptually link and differentiate trait similarity in nature, organising the huge diversity of adaptive resemblances in nature explicitly according to the processes that generate them.

The framework highlights the following key features of mimicry/masquerade systems, which must be characterized in order to allow the evolutionary processes driving them to be clearly distinguished: 1) whether or not the intended receivers of the mimic and model are shared, and therefore whether the model's traits being mimicked are cues or signals to the mimic's receiver; 2) the deceptiveness of the mimic's signal; and 3) whether the mimic manipulates receiver behaviour through advertising fitness benefits or costs. In so doing, this framework draws attention to

important gaps in our knowledge and suggests some areas where future research efforts would be revealing.

The framework identifies "rewarding" mimicry as a fourth type of mimicry that is a logical extension of the criteria used to separate the three commonly recognized mimicry forms (aggressive, Batesian and Müllerian). In rewarding mimicry, the mimic's signal is reliable and the fitness effects on the receiver of interacting with the mimic are positive. Given that researchers make a fundamental distinction between Batesian and aggressive mimicry, by the same logic, rewarding should also be differentiated from Müllerian mimicry. Currently, the best examples of rewarding mimicry are found in pollinator-plant interactions.

The reason for focussing solely on these four mimicry types, rather than the many other forms of mimicry sometimes recognised, is that these four are not restricted to a certain modality (unlike "visual" or "vocal" mimicry) or behavioural interaction (unlike "protective" or "competitive" (Rainey and Grether 2007)) or taxonomic groups (unlike "egg mimicry"). This makes them very general categories that can be applied broadly across mimetic phenomena and used to make comparisons between seemingly disparate cases of mimicry.

The "signal" versus "cue" criterion for distinguishing signal and cue mimicry allows masquerade to be categorised as a special case of cue mimicry, in which the model is inanimate/uninteresting. The same criteria used to sub-divide signal mimicry can be used to differentiate types of cue mimicry and, by extension, masquerade. This provides a clear conceptual niche for masquerade within the broader framework of mimicry, and provides internally consistent guidelines by which to explore the diversity of mimicry systems in nature.

Chapter 3:
Begging call mimicry by brood parasite nestlings: Adaptation, manipulation and development

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Jamie, G. A. & R. M. Kilner. "Begging call mimicry by brood parasite nestlings: adaptation, manipulation and development"; Book chapter in "Avian Brood Parasitism" edited by Manuel Soler (*in press*).

3.1. INTRODUCTION

Studies of avian brood parasitism have revealed a multitude of strategies employed by parasite parents and offspring to dupe host parents and allow parasites to integrate into host families (Davies 2011; Feeney et al. 2014; Rothstein 1990). Considerable attention has been given to the visual trickery of hosts by brood parasites through mimicry of host egg and chick appearance (Langmore and Spottiswoode 2012). By contrast, despite much research in the area, no equivalent examination of parasite vocal strategies for host manipulation has yet been undertaken. Such an analysis not only allows us to organise interesting natural history into a predictive theoretical framework but also provides valuable insights into the evolution of host-parasite interactions that are not evident from studying visual mimicry alone.

A key distinction between vocal and visual strategies for host manipulation is that vocal behaviour has much greater potential for plasticity. Processes, such as learning, which underpin plasticity, can generate vocal similarity between parasite and host within a matter of days rather than requiring successive generations of genetic evolution (see Chap. 27). This sets the stage for vocal similarities to arise at a pace that outstrips visual ones with potentially important evolutionary consequences (Price et al. 2003; Verzijden et al. 2012; West-Eberhard 2003; Whitman and Agrawal 2009). For example, plasticity in begging calls could allow a parasite nestling to persist in a new host environment in a single generation, exposing it to novel selection pressures and altering the course of its genetic evolution (Pfennig et al. 2010). Conversely, such plasticity could also allow the offspring of a generalist brood parasite to be successful in the nests of a range of host species without exhibiting genetic specialisation to any one host in particular.

Furthermore, the plastic nature of begging calls means that they can be used by parasites to manipulate host parents into provisioning them. Parasites can tune into the host's offspring-parent communication channel and update their displays over the course of development depending on their condition and need (Davies 2011; Kilner et al. 1999).

Finally, although begging call development can be highly plastic, calls can also develop without any influence of the host environment. Whilst some nestling brood parasites plastically develop host-specific begging calls, other species beg with calls that are unchanged by the host environment in which the parasitic nestling develops. This raises the question of why variation in begging call development exists. I examine the selection pressures underpinning mimetic vocal begging and use this to develop an adaptive framework, illustrated with empirical examples, that allows us to understand variation in begging call development for different brood parasites.

3.2. BEGGING CALL SIMILARITY BETWEEN AVIAN BROOD PARASITES AND THEIR HOSTS: A SURVEY OF THE LITERATURE

I begin by surveying the literature on begging call similarity between all avian brood parasites and their hosts (Table 3.1). This provides the empirical foundation for the conceptual sections that follow.

I collated published, and some unpublished, information on begging calls of all inter-specific avian brood parasites. For each species, I noted whether similarity has been reported between its begging calls and those of its host(s), how that similarity was assessed ("subjectively" by human comparison, or "quantitatively" by analytic comparison of sonograms) and whether that similarity is to an individual host chick or to a brood of host chicks. Additionally, I noted the level of specialisation shown by each parasite species and whether any of its hosts are known to exhibit chick discrimination or rejection. These last two parameters are included because of their importance in predicting how begging calls are likely to develop in the parasite (see Sect. 29.3).

I found that there are many gaps in knowledge of brood parasite begging calls: the begging calls of 63 of the 100 or so species of brood-parasitic birds remain entirely unknown. The largest gaps in knowledge are among the *Vidua* finches, the Asian and African cuckoos and the honeyguides. Importantly, most publications describing vocal similarity between parasite and host calls are largely anecdotal. Only eleven species of avian brood parasite have had their begging calls quantitatively compared to those of their host(s) (Table 3.1). Most studies instead have small sample sizes and assess similarity subjectively. This is problematic, as evidenced by a recent quantitative study which found no evidence for begging call mimicry of hosts by Great Spotted Cuckoos (Roldán et al. 2013), despite earlier works based on small sample sizes and subjective assessment of similarity suggesting evidence of mimicry (Mundy 1973; Redondo and Arias de Reyna 1988). Therefore, there is still work to be done validating claims of vocal similarity between parasitic and host young and providing detail about the development and information content of parasite begging calls.

Of those parasitic species whose begging calls have been described, similarity between host chick begging calls and parasitic nestling vocalisations has been reported from at least 53% (Table 3.1). Of the 12 species whose calls have been quantitatively compared to host calls, similarity has been reported from eight (Table 3.1). These findings run counter to the prevailing view in the literature. For example, in his monograph "*The Cuckoos",* Payne states that the "Begging calls of most cuckoos differ from the begging calls of their hosts" (Payne 2005a p.148). However, our literature survey reveals that, of the 25 parasitic cuckoo species for which begging calls have been described, vocal similarity between the begging calls of cuckoos and their hosts has been reported from at least 60% (Table 3.1).

The literature survey also shows that vocal similarity between avian brood parasites and their hosts has been reported from six of the seven independent transitions to parasitic lifestyles in birds (in all three transitions in cuckoos, and in each of the cowbird, finch and honeyguide transitions). The only transition lacking reports of vocal similarity is that of the black-headed duck (*Heteronetta atricapilla*) which is highly precocial and produces no begging calls (Lyon and Eadie 2013).

Thus, vocal similarity between avian brood parasites and their hosts is potentially a more widespread phenomenon than currently appreciated.

3.3 WHAT SELECTION PRESSURES UNDERPIN THE EVOLUTION OF VOCAL MIMICRY IN BROOD PARASITES?

Having documented the extent of begging call similarity between avian brood parasites and their hosts, I now examine the processes that might drive these similarities. Five hypotheses have been suggested. Two of them consider the evolution of vocal mimicry to be independent of the evolutionary interactions between brood parasites and their hosts. They suggest that similarity could be the consequence of (1) phylogenetic inertia or (2) shared ecology in the host nests (Grim 2005). However, neither is likely to be responsible for avian parasite-host begging call similarity. Phylogenetic inertia is unlikely due to the large evolutionary distances between most avian brood parasites and their hosts. Likewise although ecological factors, such as predation, have been shown to have some influence on call parameters like peak frequency and amplitude (Briskie et al. 1999) there is no evidence they can explain the majority of variation in nestling begging call structure between species. For example, closely-related nestling Estrildid finch species occupying the same habitat in southern Zambia, and presumably subject to very similar predation pressures, have highly divergent begging call structures (see Chapters 4 and 5). Therefore, whilst shared ecology may account for minor similarities in peak frequency between parasite and host, it is unlikely that it can explain major convergences in overall call structure.

I confine my attention to the remaining three hypotheses in which vocal mimicry is considered to have evolved as a direct consequence of the evolutionary interactions between brood parasites and their hosts. These are: (3) rejection of foreign chicks by hosts either through chick ejection or nest abandonment (Langmore et al. 2008); (4) the need for parasites to tune into parent-offspring communication systems in order to manipulate host parents to supply them with enough food (Davies, Kilner, and Noble 1998; Kilner et al. 1999); and (5) competition with nest mates for access to parental investment (Hauber and Kilner 2007; Pagnucco et al. 2008).

In relation to hypothesis 3, chick rejection has evolved in the hosts of several avian brood parasites (Table 3.1). These parasites exhibit begging calls that closely match those of their hosts (Langmore et al. 2008; De Márs ico et al. 2012; Ranjard et al. 2010). Therefore, it appears vocal mimicry of hosts is essential for survival in the nests of hosts exhibiting chick rejection behaviour.

Even in systems where hosts do not reject parasitic chicks outright, some parasites have evolved begging calls that closely match those of their hosts. In these cases, vocal mimicry may have evolved to tune into parent-offspring communication rules to manipulate host parents into feed them adequately (hypothesis 4). For example, nestling *Vidua* finches visually mimic the appearance of host chicks. Rather than being rejected by host parents, non-mimetic chicks are instead fed less than chicks which look like their own offspring (Schuetz 2005b). Pin-tailed (*Vidua macroura*) and Broad-tailed Paradise Whydahs (*V. obtusa*) nestlings mimic the begging calls as well as the visual appearance of their respective hosts Common Waxbill (*Estrilda astrild*) and Orange-winged Pytilia (*Pytilia afra*) (G. Jamie unpublished). Given the lack of chick rejection by these hosts, it suggests that begging call mimicry is important for soliciting sufficient levels of investment from host parents rather than to prevent removal of the parasite from the nest by host parents (Schuetz 2005b, Jamie unpublished).

In Common Cuckoos, the large size of cuckoo chicks relative to host chicks means that cuckoo chicks must tap into parent-offspring communication systems to manipulate parental provisioning (Davies et al. 1998). Here, mimicry of the whole brood is necessary for the cuckoo to signal its hunger state to the host parent and compensate for the deficient visual stimulus it provides - only one gape rather than several (Kilner et al. 1999). Some avian brood parasites, such as Shiny Cowbirds (*Molothrus bonariensis*), can also vocally manipulate host behaviour without using mimetic begging calls (Gloag and Kacelnik 2013; Tuero et al. 2015). Such situations arise where host parents are responsive to certain generic characteristics of a hungry chick rather than requiring species-specific calls.

Hypothesis 5 suggests that the evolution of vocal mimicry in nestling avian brood parasites is driven by competition with nest mates for access to parental

investment (Hauber and Kilner 2007; Pagnucco et al. 2008). This hypothesis applies to non-evictor brood parasite species (see Chap. 28) such as Viduidae finches, cowbirds, *Clamator* cuckoos, Channel-billed Cuckoo (*Scythrops novaehollandiae*) and Asian Koel (*Eudynamys scolopaceus*). Most of these have been reported to have begging calls similar to those of their hosts (Table 3.1). The traditional explanation for the evolution of trait similarity between parasites and hosts is that the parasite has converged on the characteristics of the host offspring (reviewed in Hauber and Kilner 2007). However, it is also possible that some similarity between host and parasite begging calls arises through host adaptations to resemble the parasitic chick. For example, one study experimentally parasitized Song Sparrow (*Melospiza melodia*) nests with Brown-headed Cowbird (*Molothrus ater*) chicks. Host nestlings in parasitized nests plastically altered aspects of their begging call (higher frequency, louder) so that they more closely resembled aspects of the parasite chick's begging calls (Pagnucco et al. 2008). This process of the host converging on the parasite call to compete more effectively for parental care could also explain some of the vocal similarities between host and parasite nestlings in other systems where parasites are raised alongside host young (Hauber and Kilner 2007).

3.4 THE DEVELOPMENT OF BEGGING CALLS: AN ADAPTIVE FRAMEWORK

In this final section, I present a framework to explain variation in begging call development of different brood parasite species. Our aim is to outline a verbal model that predicts when it is adaptive for begging call structure in nestling parasites to be inflexible versus phenotypically plastic. I explain the mode of begging call development by focussing on the parasite's level of specialisation and the benefits to parasitic offspring of modulating their begging calls in response to environmental cues. These benefits depend on the levels of discrimination or rejection shown by host parents against odd-sounding chicks.

Theoretical analyses can be used to predict when selection will favour plasticity versus genetically controlled development. One approach is to treat genetic and environmental cues that might influence development as competing sources of information, and identify the conditions in which one source of information is

superior to the other in yielding adaptive development (Leimar 2009). An organism's genotype is a statistical record of the selection pressures experienced by previous generations. Thus, genes are good cues when environments are stable from generation to generation - the past events that selected those genes are good predictors of selection pressures an individual will experience during their life (Leimar 2009).

I caricature the development of begging calls as either inflexible and insensitive to the particular host environment in which the brood parasitic chick is raised, or plastic and flexibly modulated by the host environment. In reality, there might be a gradient between these extremes, with the development of different call parameters being affected to a greater or lesser extent by environmental cues. During plastic development, brood parasite chicks could modulate their begging development in response to parental provisioning behaviour. Non-evictor species could instead (or as well) alter begging in response to hearing calls from host nestmates. Although there is certainly evidence of plasticity in brood parasite begging call development, the detailed mechanisms underpinning plasticity remain to be identified (Langmore et al. 2008).

3.4.1 The adaptive value of genetic cues: specialists vs. generalists

For brood-parasitic nestlings, the accuracy of genetic cues in predicting the environment in which they develop depends on the level of host specialisation exhibited by that parasite species. The more specialised the parasite on a given host species, the more likely it is that selection pressures experienced by the nestling's ancestors will match the selection pressures the nestling experiences after hatching. Therefore, to predict the adaptive value of genetic cues in influencing begging call development, we need to know the degree of host specialisation for that brood parasite species. Three categories of host specialisation can be distinguished:

i. Specialists at the species level

These species parasitize only one host species or a few closely related hosts, sharing very similar nest and nestling traits. Examples include most *Vidua* finches (Payne and Payne 2002), the Screaming Cowbird (*Molothrus rufoaxillaris*) (De Mársico et al. 2012), the Little Bronze-cuckoo (*Chalcites minutillus*) and the Shining Bronze-

cuckoo (*C. lucidus*) (Ranjard et al. 2010). For nestlings of these species, genetic cues can accurately predict the host nest in which the nestling will hatch, and the selection pressures it will consequently face. Here we should expect begging call structure to be primarily determined genetically, with minor modifications depending on the condition of the parasite nestling.

ii. Specialists at the individual level but generalists at the species level

In these species, an individual parasite targets only one host species in their lifetime but other members of that same brood parasitic species might specialise on different host species. Examples of this include the Common Cuckoo (Moksnes and Røskaft 1995) and the Cuckoo Finch (*Anomalospiza imberbis*) (Spottiswoode and Stevens 2010; Spottiswoode and Stevens 2011; Spottiswoode and Stevens 2012). Crucially, however, host specialisation is often confined to the female line, giving rise to female host races or "gentes" (Gibbs et al. 2000; Moksnes and Røskaft 1995; Spottiswoode and Stevens 2011; Spottiswoode and Stevens 2012), while males mate promiscuously across the races and exhibit no host specialisation at all (Gibbs et al. 2000; Marchetti et al. 1998). This complicates predictions of genetic cue accuracy, particularly given that sex is determined chromosomally in birds and females are the heterogametic sex (females have ZW chromosomes, males have ZZ). For female brood parasitic nestlings, genetic cues associated with the W chromosome are exclusively inherited from the mother and so can accurately predict the host nest in which the nestling will hatch, and the selection pressures it will consequently face. But this is not true for male nestlings. Infidelity in the male line with respect to past use of host species means that the Z chromosomes potentially carry mixed messages about previous host use. Two possibilities then emerge. The first is that male and female nestling parasites use different strategies to develop an adaptive begging call, with females being reliant on accurate genetic cues and males perhaps making more use of environmental cues to direct the development of their begging calls. The second possibility involves maternal effects operating in the egg before hatching. If gene products associated with the maternal W chromosome are deposited in the egg at laying then both sons and daughters could use these inherited cues to develop an adaptive begging call (see Madden and Davies 2006).

iii. Generalists at the individual level

In these species, an individual parasite will lay her eggs in the nests of multiple host species during her life. Examples of this include the Horsfield's Bronze-Cuckoo (*Chalcites basalis*) (Langmore et al. 2008), the Shiny Cowbird (*Molothrus bonariensis*) (Jaramillo and Burke 1999) and the Brown-headed Cowbird (Friedmann and Kiff 1985). The challenge for the brood parasitic nestling here is to develop an adaptive begging call when genetic cues provide little or no information about host identity. One obvious solution is to use environmental cues of host identity to develop host-specific plastic begging calls (Langmore et al. 2008). Another solution involves deploying a genetically fixed bet-hedging begging call: one that is effective enough to secure adequate care from any host species the parasitic nestling might be raised by and whose structure is unchanged by the host environment (Gloag and Kacelnik 2013).

This brief overview shows that we can predict the degree to which genetic cues might guide the development of nestling begging calls from the degree of host specialisation by the brood parasite. But it also tells us that this is not sufficient to predict the mode of begging call development in every brood parasitic species. As outlined in the previous paragraph, when individual brood parasites are generalists, for example, parasite offspring have multiple solutions available to solicit host parental investment. Therefore, to successfully predict the mode of begging call development, we need to know the scale of fitness benefits the parasitic chick stands to gain if it uses environmental cues to modify its calls. Are these gains substantial, preventing its otherwise certain death or are the benefits relatively trivial?

3.4.2 The benefits gained from using environmental cues to direct begging call development

The costs and benefits of using environmental cues to direct begging call development depend on the levels of discrimination or rejection that hosts use against foreign chicks. At one end of the continuum, are species of nestling brood parasite whose hosts can recognise cuckoo chicks as alien and reject them, either by flinging them from the nest (Sato et al. 2010; Tokue and Ueda 2010) or by abandoning them to starve to death (Langmore et al. 2003; Soler and de Neve 2012). For these parasitic

offspring, close mimicry of host young can prevent rejection and therefore death. Here there is an extremely high fitness benefit to be gained from sounding like host young. If genetic cues are too inaccurate to achieve this, then the payoffs of environmentally-induced begging call mimicry are high.

The hosts of other species of brood parasitic offspring, however, do not exhibit chick rejection, showing chick discrimination instead. In these hosts, the relationship between rates of parental provisioning in response to nestling begging displays varies depending on whether the nestling is a parasite or a host, but the parasite is never actively rejected or abandoned. For these species, the key function of brood parasitic nestling calls is to secure adequate provisioning to survive to independence. This is particularly important when brood parasitic chicks kill host offspring and so must solicit care singlehandedly from their hosts. Here the fitness benefits of environmentally-induced call mimicry depend on how host parents use nestling begging calls to refine their provisioning behaviour. For some host species it appears that only a relatively small fraction of their own offspring's call structure is used to regulate provisioning at the nest (e.g. Madden and Davies 2006). This means that the brood parasitic nestling can gain high fitness benefits by environmentally modifying its call to a small degree. It need only attune its begging call to match to these structural components of the host begging call to secure adequate provisioning. By contrast, some avian parents are particularly sensitive to features of begging calls that even their own offspring do not produce (Gloag and Kacelnik 2013). Brood parasites that can produce these sorts of calls need not mimic host nestlings at all to secure care successfully. Furthermore, their call is likely to be effective in a range of host species.

3.4.3 Predicting the mode of begging call development: an adaptive framework

I have now described two orthogonal axes for predicting the development of nestling begging calls, which are very similar to the axes described by Leimar (Leimar 2009) in predicting the adaptive value of developmental mechanisms in general. On one axis is the accuracy of genetic cues in predicting the host environment, and therefore the adaptive value of any calls produced in that environment. On the other is the fitness benefit to be gained by the parasitic nestling from environmental-induction of begging

call structure as mediated by the discriminatory behaviour of the host parents (Figure 3.1).

We can now divide Figure 3.1 into four arbitrary quadrants, within each of which we predict a particular mode of begging call development. These predictions are as follows: When genetic cue accuracy is low, because individual brood parasites are generalists, but the benefit gained from using environmental cues to modulate begging call structure is high, then we expect to see brood parasitic begging calls exhibiting phenotypic plasticity. When genetic cue accuracy is low, and the benefit gained from using environmental cues to modulate begging call structure is also low, then here we expect to see a genetically fixed bet-hedging begging call, attuned to no host species in particular but nevertheless effective at securing care from many different hosts. When genetic cue accuracy is high, because individual brood parasites specialise on a particular host species, and the benefit of environmentally modulating call structure is low, then here we expect to see a genetically fixed begging call that is insensitive to the host nest environment. Finally, when genetic cue accuracy is high and the benefits of environmentally modulating call structure are also high then here we expect to see genetically polymorphic norms of reaction. This means that individuals can modulate their begging calls to suit the host environment in which they are raised, but that there are genetic differences among chicks from different host races in the extent of call modulation in response to a common environment.

3.4.4 Testing the adaptive framework: four case studies

i) Phenotypic plasticity

I now test these ideas (Figure 3.1) with four case studies where there have been sufficient observational and experimental work to consider them within this framework. I start with the Horsfield's Bronze-Cuckoo, *Chalcites basalis*. Individual females of this species are generalists and no genetically distinct host races have been identified (Joseph et al. 2002; Langmore and Kilner 2009; Langmore et al. 2008). However, the majority of hosts used are fairy-wrens (*Malurus* spp.) and thornbills (*Acanthiza* spp.) are secondary hosts (Brooker and Brooker 1989). The default expectation for a Horsfield's bronze-cuckoo nestling is therefore that it will hatch in a fairy-wren nest. The cost to the Horsfield's bronze-cuckoo chick of making the wrong

begging call is potentially very high indeed: if it begs like a thornbill nestling in a fairy-wren nest, then it will be abandoned by its hosts to die (Langmore et al. 2008).

According to our verbal model (Figure 3.1), we should expect the Horsfield's bronze-cuckoo to exhibit a phenotypically plastic begging call: there is a low to moderate chance that genetic cues will accurately predict the host species a chick is to be raised by, and a very high fitness gain from environmental modulation of the begging call if necessary. This is indeed the case. Using cross-fostering experiments, Langmore et al. (2008) showed that the structure of the nestling cuckoo's calls is modified following parasitism by experience with their foster parents. Specifically, they found that Horsfield's Bronze Cuckoo chicks innately express begging calls that match those of their primary host, the Superb Fairy Wren (*Malurus cyaneus*). However, if the chick finds itself in a Buff-rumped Thornbill (*Acanthiza pusilla*) nest, the cuckoo starts to produce highly variable begging calls. It then relies on "social shaping" (a form of instrumental conditioning by which human parents teach toddlers to form words from their babbles) via interactions with host parents to modify its calls and mimic those produced by a Buff-rumped Thornbill (Langmore et al. 2008). Here the call repertoire is reduced to those that are most effective at eliciting feeding from host parents.

Hosts may evolve counter-strategies to limit parasitic chicks' ability to develop mimetic begging calls. Superb Fairy-Wrens have been shown to call to their eggs during incubation. After hatching, host nestlings are able to produce elements from their mother's incubation call whereas parasitic Horsfield Bronze-cuckoo nestlings are not (Colombelli-Negrel et al. 2012). This parent-specific "password", learned embryonically by host young, might help host parents detect cuckoo nestlings, although there is no direct evidence that the "password" alone is sufficient to prevent chick rejection. The suggestion is that cuckoo nestlings fail to learn the incubation call because they have a shorter incubation period than host young and are therefore exposed to the incubation call for fewer days (Colombelli-Negrel et al. 2012). (An alternative interpretation is that the "password" plays no role in preventing chick rejection, and the cuckoo is detected by hosts using other cues (Langmore et al. 2009)).

ii) Genetically fixed bet-hedging call

Female Shiny Cowbirds are also generalist brood parasites, targeting more host species than perhaps any other brood parasite (Jaramillo and Burke 1999). Genetic cues alone are therefore unlikely to predict the host species that will raise the brood parasitic chick. Consistent with our predictions (Figure 3.1), Shiny Cowbirds seemingly have a bet-hedging begging call that is effective at securing care from diverse avian parents (Gloag and Kacelnik 2013). The rate of begging has been shown to vary between host environments (after controlling for parasite chick condition and need) but call structure seems genetically fixed (Tuero et al. 2015). Additionally, when Shiny Cowbird chicks were cross fostered into the nests of Baywing (*Agelaioides badius*), their calls did not develop to resemble those made by host young (De Mársico et al. 2012). Similarly, in Brown-headed Cowbirds, there is no evidence that begging call structure varies between host environments although the average time spent begging was found to vary between host environments depending on the physical size of nest mates (Rivers 2006). However, further experimental work is needed in this species to examine how begging calls are modulated in response to the provisioning rules of different host species (Rivers 2006)

iii) Genetically fixed mimetic begging call

Unlike the Shiny Cowbird, the Screaming Cowbird is an ultra-specialist parasitising the Baywing almost exclusively (De Mársico et al. 2012). Genetic cues in this brood parasite are thus remarkably accurate in predicting the host species that will raise the brood parasitic nestling. Environmental cues are therefore redundant in this regard, and might even be a more costly way of acquiring the appropriate begging call, given that plasticity requires accurate and repeated sampling of environmental cues to be accurate (Frankenhuis and Panchanathan 2011). The benefits of environmentally-induced begging call development in this species are therefore likely to be very low. According to the model (Figure 3.1), with high genetic cue accuracy but little fitness to be gained from environmentally-induced begging calls, we should expect to see genetically fixed begging calls. Cross-fostering experiments apparently support this prediction. When Screaming Cowbird nestlings were cross-fostered to be raised by Chalk-browed Mockingbirds (*Mimus saturninus*), they retained their characteristic begging call, suggesting a strong genetic influence to call development (De Mársico et al. 2012). The same genetically fixed mimetic begging call is to be expected of the

highly specialist brood-parasitic *Vidua* finches which maintain consistent host-parasite associations over many generations through their remarkable imprinting mechanism in which females prefer to parasitise the same host species as she was raised by (Payne et al. 2000). See Chapter 4 for quantitative comparisons of *Vidua* begging calls with those of their hosts.

iv) Genetically polymorphic reaction norm

Our final case study comes from the Common Cuckoo. In this species, individual females tend to specialise in parasitizing a single host species whereas males can mate promiscuously across the female host races (Fossøy et al. 2011; Fossøy et al. 2016; Gibbs et al. 2000; Marchetti et al. 1998). Genetic cues inherited from the mother are therefore highly accurate in predicting the host that will rear the cuckoo chick, whereas genetic cues inherited from the father are less accurate – giving a moderately high level of genetic cue accuracy on average. The fitness gained from modifying the cuckoo nestling's begging call in response to environmental cues is also relatively high. Common Cuckoo nestlings are large in relation to nestlings of their hosts, they evict host young from the nest, and so single-handedly face the challenge of eliciting sustained and elevated provisioning rates with their begging call (Kilner and Davies 1999; Kilner et al. 1999). Their calls also differ among the different host species they target (Butchart et al. 2003), suggesting that calling is specifically attuned to the different host species to elicit adequate levels of care.

In short, it seems that Common Cuckoo nestlings stand to gain moderately high fitness from the environmental modification of their begging calls to suit different hosts because this allows them to procure care more effectively. We should therefore expect that them to exhibit a genetically polymorphic reaction norm (Figure 3.1): this means we might see modulation of the begging call according to the host environment, but that host races should still exhibit some differences in their calls even when raised by the same host species. This is exactly what was found in a cross-fostering study carried out on the Common Cuckoo. Here, cuckoos from eggs laid in Reed Warbler nests were transferred to Dunnock (*Prunella modularis*) nests and developed begging calls more similar to cuckoos that were naturally found in Dunnock nests. Nevertheless, they still retained some signature of their Reed Warbler host origin (Madden and Davies 2006). Thus, Reed Warbler-cuckoo chicks modulated

their begging call in a Dunnock environment, yet did not converge completely on the calls produced by Dunnock-cuckoos nestlings – just as expected with a genetically polymorphic reaction norm.

3.5 CONCLUDING REMARKS AND FUTURE DIRECTIONS

Perhaps the main contribution of this chapter is to highlight how little we know about brood parasitic begging calls, and to point the way for future work on this topic. To date, brood parasitic nestling begging calls have been described in a small number of avian brood parasites and many of these reports are based on studies with small sample sizes and without quantitative comparison of sonograms, and without consideration of how the birds themselves might hear these begging calls (Table 3.1). Much more natural history remains to be described. Importantly, though, the function of brood parasitic chick calling cannot be discerned from sonograms alone. Field experiments and comparative analyses are needed to determine how selection influences the development of the nestling begging call, as there are multiple reasons for hosts and brood parasites to share similar begging calls. Finally, I have highlighted a completely new area of research on brood parasites, by showing how they lend themselves ideally to adaptive analyses of behavioural development. I set out new theory predicting the mode of begging call development, which can be tested in future work by means of cross-fostering experiments. Interesting avenues arising from this work will be to determine whether any brood parasites learn their begging calls through interactions with nest mates, and to discover precisely how some host parents train brood parasitic nestlings to beg like host chicks.

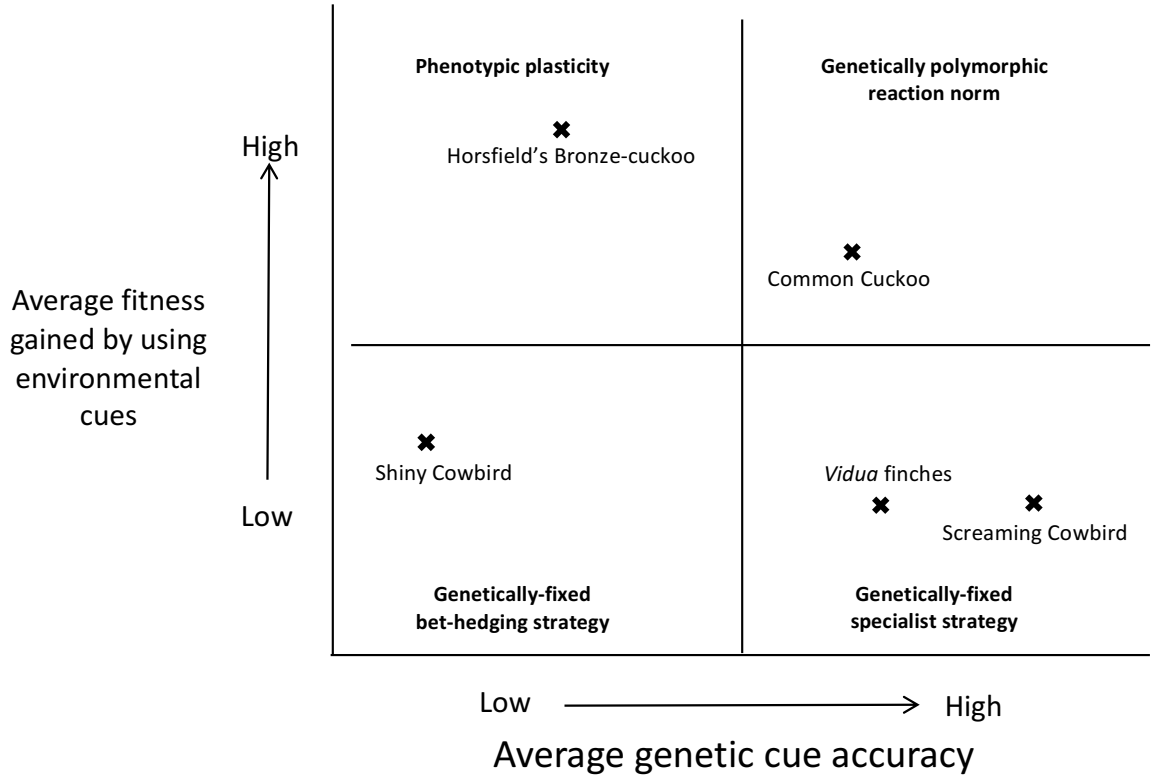


Figure 3.1. An adaptive framework to explain variation in the mode of development of brood-parasite begging calls

Table 3.1. Begging call information for those avian brood parasites whose begging calls have been described

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Cuckoos	***Cuculidae***						
American Striped Cuckoo	*Tapera naevia*	Yes (Morton and Farabaugh 1979)	To individual host chick (Morton and Farabaugh 1979)	Subjectively (Morton and Farabaugh 1979)	Generalist	Unknown	Unknown
Great Spotted Cuckoo	*Clamator glandarius*	Yes (Mundy 1973; Redondo and Arias de Reyna 1988) however, no evidence of similarity in a subsequent, more rigorous, study (Roldán et al. 2013)	To individual host chick (Mundy 1973; Redondo and Arias de Reyna 1988)	Subjectively (Mundy 1973; Redondo and Arias de Reyna 1988) and quantitatively (Roldán et al. 2013)	Specialist	Specialist	Yes (Soler et al. 1995b)
Levaillant's Cuckoo	*Clamator levaillantii*	Yes (Jubb 1952; Mundy 1973; Steyn 1973)	To individual host chick (Steyn 1973) and adult (Jubb 1952; Mundy 1973; Steyn 1973)	Subjectively (Jubb 1952; Mundy 1973; Steyn 1973)	Generalist	Unknown	Unknown

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Jacobin Cuckoo	*Clamator jacobinus*	Yes (Erritzøe et al. 2012; A. Ridley unpublished; Fry et al. 2002; Jamie and de Silva Wijeyeratne 2014)	To individual host chick (Erritzøe et al. 2012; A. Ridley unpublished; Fry et al. 2002) and adult (Jamie and Wijeyeratne 2014)	Subjectively (Erritzøe et al. 2012; A. Ridley unpublished; Fry et al. 2002; Jamie and de Silva Wijeyeratne 2014)	Generalist	Unknown	Unknown
Yellow-billed Cuckoo	*Coccyzus americanus*	No (Erritzøe et al. 2012; Payne 2005a; Potter 1980)			Generalist	Unknown	Unknown
Black-billed Cuckoo	*Coccyzus erythrophthalmus*	No (Erritzøe et al. 2012; Payne 2005a; Spencer 1943)			Generalist	Unknown	Unknown
Thick-billed Cuckoo	*Pachycoccyx audeberti*	Yes (Erritzøe et al. 2012; Fry et al. 2002; Vernon 1984)	To individual host chick (Erritzøe et al. 2012; Fry et al. 2002; Vernon 1984)		Regional specialist	Probably specialist	Unknown
Common Koel	*Eudynamys scolopaceus*	No (Gosper 1997; Payne 2005a)			Generalist	Unknown	Yes (Dewar 1907)

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Long-tailed Koel	*Urodynamis taitensis*	Yes (McLean and Waas 1987; Ranjard et al. 2010)	To individual host chick (McLean and Waas 1987; Ranjard et al. 2010)	Quantitatively (Ranjard et al. 2010) and subjectively (McLean and Waas 1987)	Generalist	Unknown	Unknown
Channel-billed Cuckoo	*Scythrops novaehollandiae*	Yes (Courtney 1967; Lord 1956)	To individual host chick (Courtney 1967; Lord 1956)	Subjectively (Courtney 1967; Lord 1956)	Generalist	Unknown	Unknown
Dideric Cuckoo	*Chrysoccoccyx caprius*	No – but does vary non-mimetically between host races (Reed 1968)			Generalist	Gentes	Unknown
Klaas's Cuckoo	*Chrysoccoccyx klaas*	No (Erritzøe et al. 2012; Payne 2005a; Skead 1995)			Generalist	Unknown	Unknown

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Horsfield's Bronze Cuckoo	*Chalcites basalis*	Yes (Langmore et al. 2008)	To individual host chick (Langmore et al. 2008)	Quantitatively (Langmore et al. 2008)	Generalist	Generalist	Chick rejection (Langmore et al. 2003)
Shining Bronze Cuckoo	*Chalcites lucidus*	Yes (Anderson et al. 2009; McLean and Waas 1987; Ranjard et al. 2010)	To individual host chick (Anderson et al. 2009; McLean and Waas 1987; Ranjard et al. 2010)	Quantitatively (Anderson et al. 2009; Ranjard et al. 2010) and subjectively (McLean and Waas 1987)	Specialist	Unknown	Chick rejection (Langmore et al. 2003; Langmore et al. 2011; Sato et al. 2015)
Little Bronze Cuckoo	*Chalcites minutillus*	Yes (N. Langmore unpublished)	To individual host chick (N. Langmore unpublished)	Subjectively (N. Langmore unpublished)	Specialist	Specialist	Chick rejection (Sato et al. 2010; Tokue and Ueda 2010)
Black-eared Cuckoo	*Misocalius osculans*	Yes (Erritzøe et al. 2012)	To individual host chick (Erritzøe et al. 2012)	Subjectively (Erritzøe et al. 2012)	Generalist	Unknown	Unknown

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Pallid Cuckoo	*Cacomantis pallidus*	Yes (Courtney 1967; Serventy and Whittell 1962)	To individual host chick (Courtney 1967; Serventy and Whittell 1962)	Subjectively (Courtney 1967; Serventy and Whittell 1962)	Generalist	Unknown	Unknown
Fan-tailed Cuckoo	*Cacomantis flabelliformis*	No (Clunie 1973; Erritzøe et al. 2012)			Generalist	Unknown	Unknown
Rusty-breasted Cuckoo	*Cacomantis sepulcralis*	No (Grim 2008)			Generalist	Unknown	Unknown
Common Hawk-cuckoo	*Hierococcyx varius*	Yes (Ali and Whistler 1936)	To individual host chick (Ali and Whistler 1936)	Subjectively (Ali and Whistler 1936)	Generalist	Unknown	Unknown
Black Cuckoo	*Cuculus clamosus*	No (Payne 2005a; Skead 1946)			Generalist	Unknown	Unknown
Red-chested Cuckoo	*Cuculus solitarius*	No (Payne 2005a; Salewski and Grafe 1999)			Generalist	Unknown	Unknown

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Indian Cuckoo	*Cuculus micropterus*	Yes (Erritzøe et al. 2012; Payne 2005a)	To individual host chick (Erritzøe et al. 2012; Payne 2005a)	Subjectively (Erritzøe et al. 2012; Payne 2005a)	Generalist	Unknown	Unknown
African Cuckoo	*Cuculus gularis*	No (Fry et al. 2002)			Generalist	Unknown	Unknown
Common Cuckoo	*Cuculus canorus*	Yes for some hosts (Butchart et al. 2003; Davies et al. 1998; Erritzøe et al. 2012; Madden and Davies 2006; Payne 2005a)	To brood of host chicks (Davies et al. 1998)	Quantitatively (Butchart et al. 2003; Davies et al. 1998; Madden and Davies 2006)	Generalist	Gentes	Yes, in one population but not others (Davies et al. 1998; Grim et al. 2003)
Honeyguides	**Indicatoridae**						
Eastern Green-backed Honeyguide	*Prodotiscus zambesiae*	No (Short and Horne 2001; Vernon 1987)			Generalist	Unknown	Unknown
Lesser Honeyguide	*Indicator minor*	No (Short and Horne 2001)			Generalist	Unknown	Unknown

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Scaly-throated Honeyguide	*Indicator variegatus*	No (Short and Horne 2001)					Unknown
Greater Honeyguide	*Indicator indicator*	Yes (Jubb 1966)	To brood of host chicks (Jubb 1966)	Subjectively (Jubb 1966)	Generalist	Generalist	Unknown
Cowbirds	**Icteridae**						
Screaming Cowbird	*Molothrus rufoaxillaris*	Yes (De Mársico et al. 2012)	To individual host chick (De Mársico et al. 2012)	Quantitatively (De Mársico et al. 2012)	Specialist	Specialist	Chick rejection (De Mársico et al. 2012)
Brown-headed Cowbird	*Molothrus ater*	No (Dearborn 1998; Rivers 2006)		Quantitatively (Rivers 2006)	Generalist	Generalist	No evidence of discrimination (Dearborn 1998; Rivers et al. 2010)
Shiny Cowbird	*Molothrus bonariensis*	No (Gloag and Kacelnik 2013; Tuero et al. 2015)		Quantitatively (Gloag and Kacelnik 2013; Tuero et al. 2015)	Generalist	Generalist	Yes, by at least some hosts (Dearborn and Lichtenstein 2002; Lichtenstein 2001)

English Name	Scientific Name	Parasite begging calls			Parasite specialization level (from Erritzøe et al. 2012)		Hosts known to show discrimination against parasite nestlings? (Grim 2006)
		Reported similarity to host's?	Nature of similarity	How similarity assessed	Species-level	Individual-level	
Parasitic finches	**Viduidae**						
Village Indigobird	*Vidua chalybeata*	No (Payne and Payne 2002)		Quantitatively (Payne and Payne 2002)	Specialist	Specialist	Yes (Payne et al. 2001)
Purple Indigobird	*Vidua purpurascens*	Yes (G. Jamie, Chapter 4)	To individual host chick (G. Jamie, Chapter 4)	Quantitatively (G. Jamie, Chapter 4)	Specialist	Specialist	Unknown
Pin-tailed Whydah	*Vidua macroura*	Yes (G. Jamie, Chapter 4)	To individual host chick (G. Jamie, Chapter 4)	Quantitatively (G. Jamie, Chapter 4)	Prob. regional specialist	Specialist	Yes (Schuetz 2005b)
Broad-tailed Paradise Whydah	*Vidua obtusa*	Yes (G. Jamie, Chapter 4)	To individual host chick (G. Jamie, Chapter 4)	Quantitatively (G. Jamie, Chapter 4)	Specialist	Specialist	Unknown
Cuckoo Finch	*Anomalospiza imberbis*	No (C. Spottiswoode unpublished)		Subjectively (C. Spottiswoode unpublished)	Generalist	Specialist	Yes (Spottiswoode et al. 2012)

Chapter 4: *Multimodal mimicry of hosts by* Vidua *finches*

Chapter 4: Multimodal mimicry of hosts by *Vidua* finches

4.1 INTRODUCTION

Parasitic behaviour is widespread in the natural world (Poulin 1997). A common problem faced by parasites is how to avoid detection by the species they are exploiting. One solution is to mimic the characteristics of the host, allowing parasites to draw on host resources without stimulating a defensive response. For example, chemical mimicry is employed by parasitic butterflies whose caterpillars copy the hydrocarbons of the ant species from whom they receive parental care (Akino et al. 1999). Similarly, parasitic species of bumble bee mimic the hydrocarbons of the specific host species of bumblebee they exploit (Martin et al. 2010).

Perhaps the best examples of mimicry to dupe hosts are found in avian brood parasites. Here, mimicry is a common strategy to evade host defences and integrate into the host's life cycle (Davies 2011). This occurs at the egg stage, where parasites lay eggs that match the appearance of their host's eggs (Brooke and Davies 1988; Spottiswoode and Stevens 2010), and at the chick stage, where some parasite nestlings match the appearance (De Márcico et al. 2012; Langmore et al. 2011; Nicolai 1964) and begging calls (see references in Chapter 3) of their hosts.

The indigobirds and whydahs (genus *Vidua*) of sub-Saharan Africa are host-specific brood parasites of finches in the estrildid family. Estrildid finches show extreme between-species diversity in nestling begging displays (Payne 2005b, Chapter 6) and *Vidua* nestlings are known to mimic the mouth markings of their hosts (Neunzig 1929; Nicolai 1964). However, the mouth markings of hosts and parasites have never been compared quantitatively. Additionally, other aspects of host begging display (such as begging calls and head movements) have also never been quantitatively compared with those of their hosts. In this chapter, I have two main aims: (i) to quantify the mimicry of host begging displays by *Vidua* finches; and (ii) to test whether the mimicry of host begging displays by *Vidua* is imperfect (i.e. that there are consistent differences between *Vidua* and their host's begging displays). The

begging display traits I am investigating are the nestling mouth markings, begging calls and head movements.

To increase the reliability of their discrimination, hosts sometimes use multiple lines of evidence to differentiate host from non-host (e.g. Spottiswoode and Stevens 2010). This means mimics can be selected to converge on model phenotypes in more than one modality. For example, some orchids mimic both the appearance and the smell of female insects to lure male pollinators (Jersakova et al. 2006). Many ant-mimicking spiders copy both the morphology and the movements of their ant models (Shamble et al. 2017), as do some hoverflies of their hymenopteran models (Penney et al. 2014). Only one avian brood parasite system has so far been shown to exhibit both visual and vocal mimicry of host nestlings, namely the Screaming Cowbird (*Molothrus rufoaxillaris*) which mimics its host the Baywing (*Agelaioides badius*) (De Mársico et al. 2012).

Visual mimicry of host mouth markings by nestling Vidua finches

The mimicry of mouth markings is important in soliciting care from their foster parents, as chicks with different or even slightly altered mouth markings receive less food (Payne et al. 2001; Schuetz 2005b, Chapter 5 of this thesis). While it has been known for many decades that *Vidua* mimic the mouth markings of their host species (e.g. Neunzig 1929; Nicolai 1964), this similarity has never been quantified.

Most birds perceive colour differently to humans (Burkhardt 1989). Passerines are tetrachromats possessing four types of cone in their retinas. In most passerines, including estrildid finches, this includes one that is sensitive to UV light (Hart et al. 2000a; Odeen et al. 2011). This means that any colour comparisons between host and parasite nestling appearance must consider these signals from an avian visual perspective. In this chapter, I provide the first quantitative comparisons of *Vidua* and host mouth marking patterns.

Vocal mimicry of hosts by nestling Vidua finches

In contrast to mouth marking mimicry, the begging calls of most *Vidua* have previously been thought to generally not match those of their hosts (Payne and Payne 2002). Prior to the work presented in this thesis chapter, the begging calls of only one

species of indigobird, the Village Indigobird (*V. chalybeata*) had ever been described, and these were judged to differ from those of its Red-billed Firefinch (*Lagonosticta senegala*) host (Payne et al. 1998). Jürgen Nicolai also described the begging calls for two species of whydah, the Straw-tailed Whydah (*V. fischeri*) (Nicolai 1973) and the Long-tailed Paradise Whydah (*Vidua paradiseae*) (Nicolai 1969). Of these, Nicolai suggested that the begging calls of young nestling Long-tailed Paradise Whydahs were initially similar to those of its host, Melba Finch (*Pytilia melba*), but that the calls of host and parasite diverged as the they grew older and differed from one another by the time of fledging (Nicolai 1969; Payne and Payne 2002). However, none of these studies were quantitative and were instead only based on subjective assessment of call similarity.

Begging call mimicry has been inferred for a few other *Vidua* species from recording the songs of adult males. Adult male *Vidua* imitate host vocalisations as part of their sexual display, including host begging calls (Nicolai 1964; Payne and Payne 2002). However, is not necessarily safe to assume that just because a *Vidua* imitates host begging calls in sexual display as an adult, that it also imitates host begging calls as a nestling to solicit investment from host parents. Therefore, it is necessary to record *Vidua* calls directly as nestlings.

Given that *Vidua* are highly host-specific, and given the importance of mouth marking mimicry in obtaining food from parents, it is plausible to expect that begging calls would also be selected to be mimetic, since they are another crucial aspect of begging displays. Mimicry of host begging calls has been reported from each of the six independent origins of parasitic behaviour involving altricial young (see Chapter 3), but has not yet been demonstrated quantitatively in the parasitic finches. In this chapter, I describe the begging calls of three *Vidua* species for the first time: Pin-tailed Whydah (*V. macroura*), Broad-tailed Paradise Whydah (*V. obtusa*) and Purple Indigobird (*V. purpurascens*) and provide the first quantitative evidence that each of these species mimic the begging calls of their respective hosts.

Postural mimicry of hosts by nestling Vidua

A third dimension of nestling estrildid begging displays are the head movements made by young as they beg (Nicolai 1964; Payne and Payne 2002). These vary from

species to species, with some moving their head left-to-right, some twisting clockwise and anti-clockwise, and at least one (the Common Waxbill, *Common Waxbill*) not moving its head at all. The possibility of postural mimicry between *Vidua* and their hosts has been even less well explored than that of begging call mimicry. In this chapter, I describe the postural head movements of three species of *Vidua* parasite and compare them to those of their hosts.

Imperfect mimicry of hosts: adaptation, constraint or weak selection?

When parasites mimic their hosts, the resemblance is not always exact. Sometimes there are distinct and consistent differences between host and parasite phenotypes. An example of this is the eggs of the brood-parasitic Cuckoo Finch (*Anomalospiza imberbis*). Despite otherwise closely mimicking the colour and pattern of its host, the Tawny-flanked Prinia (*Prinia subflava*), Cuckoo Finch eggs always lack the broad dark squiggles present on the prinia egg (Spottiswoode and Stevens 2010).

There are many examples in nature of imperfect mimicry, including hoverflies mimicking hymenopteran models (Penney et al. 2012) and orchids mimicking the sexual pheromones of female bees (Vereecken and Schiestl 2008). There have been many hypotheses put forward for the evolution of imperfect mimicry (Edmunds 2000; Johnstone 2002; Sherratt 2002). All the hypotheses fall into one of four broad categories: (i) selection is not strong enough to lead to the evolution of more accurate mimicry ("weak selection"); (ii) there are genetic or developmental constraints that prevent the parasite from evolving more accurate mimicry, despite the selection pressure being present ("constraint"); (iii) parasite signals represent an enhanced or improved version of the model's trait such that the mimic is even more effective at manipulating receiver behaviour than the model is ("superstimulus"); (iv) the selective pressure and the genetic/developmental potential are present, but there has not yet been enough time for the mimic to converge on the host more precisely ("evolutionary lag").

The super-stimulus hypothesis has been tested in several other avian brood parasite species. Evidence in favour of the hypothesis has come from Great Spotted Cuckoos (*Clamator glandarius*) which were found to be preferentially fed by host parents compared to host chicks (Soler et al. 1995a). Here, both the large size and the

conspicuous gape papillae of the cuckoo chick served to elicit more feeding from parents (Soler et al. 1995a). In the Common Cuckoo (*Cuculus canorus*), chicks have been shown to make begging calls which imitate the sound of an entire brood of host chicks, stimulating parents to feed them more than they otherwise would a single host chick (Davies et al. 1998). By contrast, a study on Brown-headed Cowbirds (*Molothrus ater*) found that parasitic cowbird chicks got more food from parents because they were able to physically dominate their host nestmates rather than because they provided a superstimulus to host parents (Lichtenstein and Sealy 1998).

If the super-stimulus hypothesis were true in *Vidua* finches, we might expect parasite mouth markings to be more conspicuous in appearance than host mouth markings. Specifically, nestling mouths could be more conspicuous if they had (i) higher luminance of conspicuous features, or (ii) higher levels of contrast in colour between adjacent mouth features and (iii) larger spots relative to the entire mouth area. I test these predictions of the super-stimulus hypothesis.

Throughout this chapter the focus is on three host-parasite pairs: 1) Common Waxbill (host) and its parasite the Pin-tailed Whydah (*V. macroura*); 2) Jameson's Firefinch (*Lagonosticta rhodpareia*) and its parasite the Purple Indigobird (*V. purpurascens*); and 3) Orange-winged Pytilia (*Pytilia afra*) and its parasite the Broad-tailed Paradise Whydah (*V. obtusa*). In addition, I document the mouth markings and begging calls of the nestlings of five other species of estrildid finch occurring sympatrically at our field site in southern Zambia to situate the begging displays of *Vidua* and their hosts in a broader context.

4.2 METHODS

Fieldwork

During January–May 2014, 2015, 2016 and 2017, I carried out transfer experiments within an area of about 40 km^2 on and around Musumanene and Semahwa Farms (centred on 16°47′S, 26°54′E) in the Choma District of southern Zambia. The habitat is a mixture of miombo woodland, grassland and agricultural fields.

Visual mimicry

Photographing Vidua and estrildid nestling mouths

Eggs were taken from nests and placed in a Brinsea Octagon 20 Advance EX Incubator at 36.7°C and 60% humidity. Photographs were taken on chicks freshly hatched from the incubator. The chick was held such that it bit on the edge of a prism (PEF2525 Equilateral prism, UV fused siliaca, 25 x 25 mm aperture (Figure 4.1). This allowed the angular surface of the chick's mouth to be projected onto the flat surface opposite one of the prism's edges. A wooden block was made to secure the prism (Figure 4.1). The block also had a ledge underneath onto which a certified 40% Spectralon grey standard (Labsphere, Congleton, UK) could be placed. Photos were taken with a Nikon D7000 camera and a Micro-Nikkor 105 mm lens. The camera had undergone a quartz conversion (Advanced Camera Services, Norfolk) to allow sensitivity to both human-visible and UV wavelengths. This involved replacing the UV and infrared (IR) blocking filter with a quartz sheet to enable analysis through the avian visual spectrum. The camera was placed on a tripod and pointed vertically down onto the flat surface of the prism at approximately 50cm distance. The chick was gently held between thumb and forefinger as it bit on the prism. For each individual nestling, two photos were taken, each with a different filter. UV photographs were taken with a Baader UV pass filter (transmitting 320-380 nm). Human-visible photos were taken with a Baader UV-IR blocking filter (transmitting 420-680 nm). For each shot the camera was set to f13 and the shutter speed varied to get the best exposure. The flash (Metz 76 MZ-5 digital) was attached to the camera body and had been modified to emit both visible and UV light. Advanced Camera Services initially removed the UV filter to allow UV light to be emitted. Jolyon Troscianko subsequently removed the visible filter on the converted flash and replaced it with a quartz filter to allow the visible light to be transmitted too. The flash was set to under-expose by 3 stops for the "visible" images, and to over-expose by 3 stops for the "UV" image. An ISO of 400 was used for all photos and images were taken in RAW (NEF) format. These settings were found to give the best overall exposure. All images were taken indoors with windows closed and the room light switched off to minimise sources of light other than those from the flash.

Figure 4.1. Photographing the mouth markings of nestling finches. The chick is held gently and allowed to bite on the apex of a prism and the photograph is taken from directly above. The grey standard is supported in a gap in the wooden block and is visible through the prism alongside the chick's mouth.

Analysing photographs

Pattern analysis

Quantification of the spot pattern on the upper palate was carried out using the R package patternize by Steven van Belleghem (Van Belleghem et al. 2017). Patternize allows variation in colour patterns to be quantified from images. The first step was to allow the package to identify homologous regions of the mouth in each photograph. This was achieved by manually placing seven landmarks on reference points around the mouth (Figure 4.2). For simplicity, only "visible" images were used in this analysis and not the "UV" images. Having identified landmarks in each image, the images were aligned to an arbitrarily chosen reference image. This transformation involves both uniform changes (affecting each point in the image equally) such as translation, rotation, scaling and skewing (2001), as well as non-uniform changes (such as the thin plate spline transformation) in which different parts of the image are bent unequally (Duchon 1976). This allowed patterns to be compared among images even if there were slight differences in the distances between camera and chick and in the positioning of the chick within the image.

Pattern identification in each image was performed by selecting pixels within a specified colour range. Thresholds could be adjusted in the red, green and blue channels. To extract the black upper palate markings, the thresholds were manually adjusted for each image and their success at extracting black patterns assessed. The process was repeated until the black markings were extracted as accurately as possible. It was necessary to manually adjust the thresholds because the lighting sometimes varied between images. Regions that had been extracted due to shading were manually removed from the selection.

The output of the colour pattern analysis is a table of pixel coordinates which are assigned either a "1" (have the colour of interest) or a "0" (lack the colour of interest). The variance-covariance matrix obtained from this binary matrix is suitable for principal component analysis (PCA). This allows the main variations in colour pattern between groups to be visualised. In the context of *Vidua* and estrildid mouth markings, the position of black markings on the upper palate could be quantified and compared between species.

To compare spot size between hosts and parasites, the number of pixels in the standardised images that each of the upper palate spots contained was calculated for every individual. Comparisons were performed with Kruskal-Wallis tests.

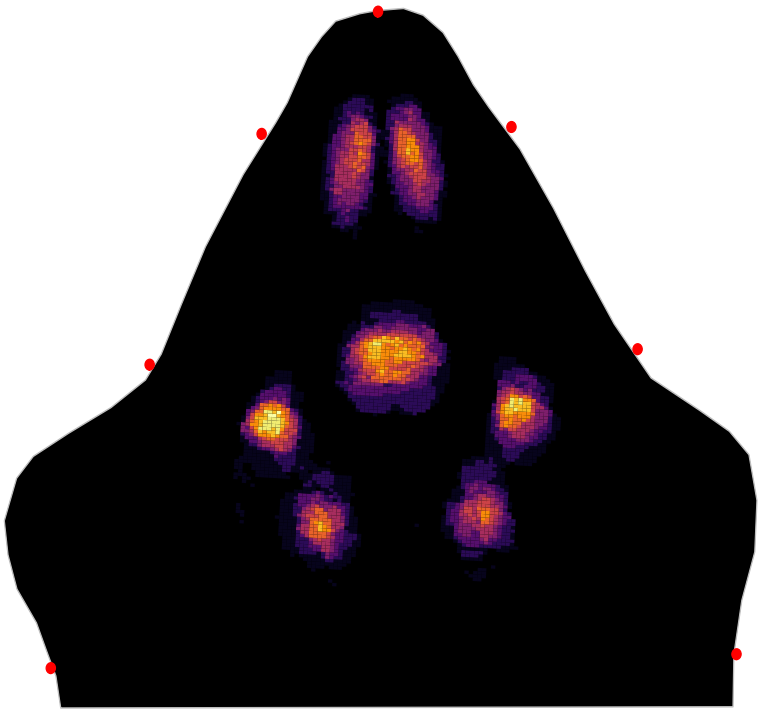


Figure 4.2. Landmarks (red dots) placed along the edge of the upper palate used to transform the mouth marking photographs and allow comparisons between images for pattern analysis using the R package patternize (Van Belleghem et al. 2017). Here a Common Waxbill mouth is shown as an example.

Colour analysis

To investigate whether parasite and host mouths showed consistent differences in colour from an avian visual perspective, colours of corresponding regions of host and parasite mouths were analysed using the Multispectral Image plugin (Troscianko and Stevens 2015) for ImageJ (Schneider et al. 2012). Both "visible" and "UV" images were used in this analysis. Colour analysis was only carried out on the Pin-tailed Whydah – Common Waxbill parasite – host pair as this was the only pairing with sufficient sample sizes.

For every individual photographed, regions of interest (ROIs) were selected manually in ImageJ for the visible and UV images separately (Figure 4.3). ROIs were: 1) gape flange, white outer edge (left and right); 2) gape flange, black centre (left and right); 3) medial palate spot; 4) lateral palate spot (left and right); 5) mediolateral palate spot (left and right); 6) black mark at distal tip of upper beak; 7) tongue marking (left and right). Grey standards were also highlighted in each image. It was not possible to align UV and visible images exactly as the

chick moved slightly between each photo being taken. Instead, raw linear pixel values were extracted for each ROI separately for UV and visible images. Once raw linear pixel values had been obtained for ROIs in UV and visible images, these were put through a model of avian vision to obtain "cone-capture" values. The visual model of the passerine whose vision has been most studied, the Blue Tit (*Cyanistes caeruleus*), was used (Hart et al. 2000b) . Most higher passerines are thought to differ relatively little in their spectral sensitivity and Blue Tit vision is known to be similar to that found in estrildid finches, with both possessing UV sensitive short-wave opsin cones (Hart et al. 2000a; Odeen and Hastad 2003; Odeen et al. 2011).

To measure luminance and colour contrast between different internal mouth features, Just Noticeable Differences (JNDs) were calculated between adjacent mouth marking features. JNDs are a measure of colour differences between to objects in predicted discrimination values, where values of less than 1.00 imply the two objects are not discriminable to an observer with that visual system (Vorobyev and Osorio 1998). The greater the JND value, the greater the contrast in the appearance of those two colours to the receiver. This approach is commonly used in studies of animal colouration (for a review see Renoult et al. 2017).

Luminance JNDs were calculated between the white outer gape flange and the inner black section of the gape in hosts and parasites (see Figure 4.3). These features were chosen as they are large, conspicuous structures at the edges of the chick's mouth that are likely to be obvious to a parent when feeding. They are also structures which differ drastically between estrildid species (see Chapter 6) and so are likely to be of importance to parental discriminatory behaviour. Additionally, I calculated colour JNDs between the black upper palate spots and the adjacent background palate colour for Pin-tailed Whydahs and Common Waxbills. As with the gape flanges, the upper palate spots are well exposed to parents when the chick is begging and show a lot of variation across the estrildid family tree (see Chapter 6). These contrasts between mouth characters were compared for hosts and parasites using Kruskal-Wallis tests.

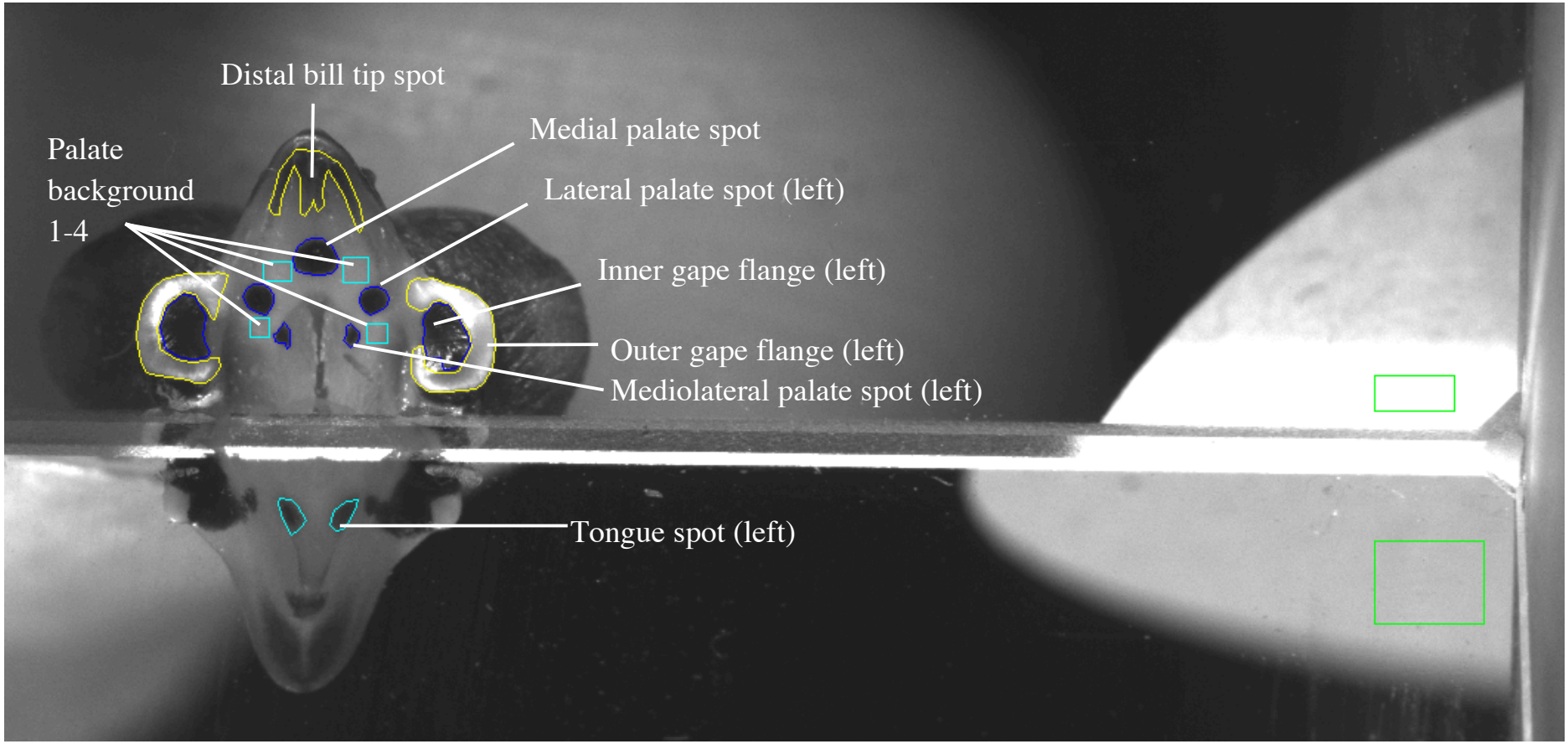


Figure 4.3. Regions of Interest (ROIs) highlighted for colour and luminance analysis using the Multispectral Image analysis plugin in ImageJ (Troscianko et al. 2015). For every ROI only labelled on the left-hand side, the right-hand side feature was also included in the analysis. The two green rectangles on the right of the image are the upper and lower prism grey standards. The upper prism grey standard was used for all ROIs except tongue spots which used the grey standard reflected on the lower facet of the prism.

Vocal mimicry

Recording nestling begging calls

Chicks were removed from the nest and placed in an artificial nest inside a box. The artificial nest consists of an orange plastic bowl used as a nest platform in aviculture, tightly lined with nesting material from abandoned estrildid nests. Chicks were left in the fake nest with the box closed (or partially closed to allow air in and prevent overheating) for a few minutes to allow acclimation. To stimulate begging, the chick was tapped gently with forceps on the bill. To initially stimulate begging, the tapping was more rapid than that which was subsequently used to sustain begging. Initial taps with the forceps would often lead to a slight and then complete opening of the mouth. Tapping inside the mouth would often elicit vocalizations. Once the bird had started begging, the bird's beak would be tapped gently approximately once every 3 seconds. For some birds this rate of tapping was too slow to maintain begging and the bird would go quiet. In such cases I increased the frequency of taps (but made a note of this in the recording; it is also evident from videos).

Recording begging recordings in an artificial nest and stimulating begging manually has the disadvantage of not recording a natural parent-offspring begging interaction. However, I decided to record chicks in a fake nest rather than inside the natural nest for the following reasons. First, host nest mates were often present alongside the transferred chick. This would make isolating which calls came from which chick very difficult if a microphone was strapped to the bird's nest and a natural parent-offspring encounter recorded. The calls from multiple chicks would overlap, making it difficult to extract call parameters from a single call. Second, estrildid host parents visit the nest only infrequently (around once per hour). This would make it logistically difficult to get recordings as microphones would have had to be strapped to nests for long periods of time before adequate material was obtained.

Recordings were made using a tie-clip microphone (Audio-Technica ATR35s) (2014 (part), 2015, 2016 and 2017 seasons or a Sennehiser ME-66 shotgun microphone (2014 (part) season) held by hand approximately 3 cm away from the focal bird's mouth. Files were recorded in WAV format on a Tascam DR-05 portable recorder. Recordings were made for around 2 minutes or until sufficient amounts of begging had been obtained (at least 10 seconds of continuous begging where possible). Sonograms were produced and analysed using the default settings in Raven Pro (Bioacoustic Research Program 2014).

Testing for mimicry in Vidua *begging calls*

Two types of approach were taken to test the hypothesis that nestling *Vidua* mimic the begging calls of their hosts.

Multinomial logistic regression/Discriminant function analysis

In the first approach, eight parameters were extracted from each call: frequency bandwidth, call duration, peak frequency, centre frequency, minimum frequency, maximum frequency, energy and aggregate entropy. These are parameters that have been used previously to characterise the vocalisations of birds, particularly to compare the begging calls of avian brood parasites and their hosts (Anderson et al. 2009; De Mársico et al. 2012; Langmore et al. 2008).

A multinomial logistic regression (MLR) model was used with species identity as the response variable and the 8 call parameters as the explanatory variables. Initially, only host species were used in the MLR as "training data". This created a function built from the 8 parameters which best separates the begging calls of each host species. Subsequently, parasite begging calls were fed into this formula and were thus assigned a host species that their call was most similar to. Begging call data from 8 species of locally occurring estrildid finches (including hosts and non-hosts) were entered into a multinomial logistic regression model to generate a classification function. These training data included calls from 8 Common Waxbill, 1 African Quailfinch, 4 Blue Waxbill, 2 Bronze Mannikin, 2 Jameson's Firefinch, 2 Melba Finch, 3 Orange-winged Pytilia and 2 Zebra Waxbill individuals, each from a different nest.

To maximise the discriminatory ability of the MLR, individual call notes, rather than means for individuals, were used as input data points. This allowed the maximum amount of data to be used in the creation of the classification function. It also means that the model is exposed to parameter values from actual calls rather than to abstract "mean calls". 10 call notes from each parasite individual were used as test data. I tested five Pin-tailed Whydah and two Broad-tailed Paradise Whydah individuals, and 1 Purple Indigobird individual.

The measure of mimicry for each parasite individual was what proportion of the 10 input calls were assigned to the correct host. Each parasite individual is given this "proportion correct" score. If the mean of these scores was significantly greater than that

expected by chance, then it suggests that parasites match the calls of their hosts better than the other sympatric estrildid species. By establishing a "proportion correct" score for each individual, the problem of pseudoreplication is overcome. However, by entering individual call notes rather than individual-level means, the accuracy of the classification function is increased.

Pin-tailed Whydahs make multiple begging call types through development. For a detailed breakdown of each of these call types and the stages in development at which they are made, see Chapter 5. One call type (termed call type 4 in Chapter 5) is made only by nestlings in mid to late development. It is a distinctive, two note "we-chee" call (Figure 4.13). In this chapter and in chapter 5, the first part of this call is termed call type 4a and the second part call type 4b. Common Waxbill nestlings also make a similar two-note call in mid to late development.

To simplify the analysis, only the type 4 calls of Pin-tailed Whydahs and Common Waxbills are included in the analysis. Similarly, other estrildid species also show different call types earlier in development. Again, only calls made by other estrildid nestlings in mid to late development were included. Mid-development stage was characterised as being the point at which the primaries had irrupted from pin. This has been used as an indicator of developmental stage in other studies of brood parasite begging (Briskie et al. 1999; Ranjard et al. 2010). Three of the five Pin-tailed Whydah chicks used in the analysis of begging call mimicry had been raised in the nest of a Blue Waxbill and not the natural Common Waxbill nest. These chicks had been transferred to Blue Waxbill nests as part of transfer experiments outlined in Chapter 5. It was considered justifiable to include these in the analysis of Pin-tailed Whydah begging call mimicry because the type 4a and 4b calls could still easily be identified in Pin-tailed Whydahs raised in the foreign host environment. Additionally, if the calls of Pin-tailed Whydahs raised in a Blue Waxbill nest are still assigned as most similar to Common Waxbill calls by the model, this would suggest that the Pin-tailed Whydah begging call mimicry is largely innate and not dependent on interactions with its specific host.

A discriminant function analysis (DFA) was also used as an alternative approach to the multinomial logistic regression. Again, host calls were initially entered as training data, and parasite calls subsequently entered as testing data. DFA and MLR are similar approaches but MLR has less restrictive assumptions than DFA. However, when DFA's assumptions are

met, it is a more powerful approach (citation). DFA can be used with smaller sample sizes than MLR, and is more accurate when sample sizes are equal. In my dataset, sample sizes were often quite small (< 6 individuals), supporting the use of a DFA over MLR. However, sample sizes varied between species suggesting that MLR might be more appropriate. For this reason both approaches were used to see if the conclusions were robust across methods.

MLR was implemented using the *multinom* function from the R package nnet (Venables and Ripley 2002). DFA was done using the *lda* function from the R package MASS ((Venables and Ripley 2002). The observed versus expected percentages were compared using the prop.test function in R base stats package (R Development Core Team 2017).

Human assessment of similarity from sonograms

The second type of approach was to use human assessment of sonogram similarity. The human brain is very good at detecting patterns (Ripley 1996). In addition to looking at the structure of individual notes (which was quantified in the MLR and DFA analyses), humans can also easily interpret higher level patterns in overall syntax structure. For example, humans can quickly assess whether call type A is usually followed by call type B, or whether other higher level patterns exist (Schwab and Nusbaum 1986). These could, in theory, be quantified and entered into a regression model, but the human approach is quicker and easier. Human visual assessment of sonograms has previously been used successfully to examine mimicry of heterospecifics in the alarm calls of Brown Thornbills (*Acanthiza pusilla*) (Igic and Magrath 2013).

Humans (n = 10; a mixture of colleagues in the Department of Zoology and friends from Cambridge who were blind to the hypothesis under test) were presented with 8 reference sonograms laid in front of them in a 2x4 grid. They were then given 18 sonograms and asked to find the closest match in appearance between each sonogram in their hand and one of the 8 reference sonograms. Each of the 8 reference sonograms displayed the nestling begging call of a different estrildid finch species that occurs at the field site in Zambia, and the participants were not told which species was which. The order in which the 8 reference sonograms was laid out in front of the participant was randomised for each trial. The single sonogram that was used as the reference for each of the 8 estrildid species was also randomised for each trial. Additionally, the 18 sonograms to be matched were shuffled

between trials. This guards against any bias in the participants' assignments with respect to the order in which sonograms were presented, or with respect to any tendency to match reference sonograms to certain positions of the grid, or to make particular comparisons between adjacent sonograms.

Estrildid begging calls change with age, and so to compare like with like and simplify the analysis, only sonograms of chicks in mid to late development stage (after the primaries had irrupted from pin) were presented. The sonograms were on the same scale, with a one second interval on x-axis and 0–23 kHz frequency range on y-axis. The eight estrildid finch species whose begging calls were presented on the reference sonograms were African Quailfinch (*Ortygospzia atricollis*), Bronze Mannikin (*Spermenstes cucullatus*), Jameson's Firefinch (*Lagonosticta rhodopareia*), Melba Finch (*Pytilia melba*), Orange-winged Pytilia (*P. afra*), Zebra Waxbill (*Amandava subflava*), Blue Waxbill (*Uraeginthus angolensis*) and Common Waxbill (*Common Waxbill*).

The 18 sonograms the participants were asked to match contained a mixture of estrildid finch and *Vidua* begging calls. This comprised 7 *Vidua* recordings and 11 estrildid recordings. The *Vidua* recordings to match were 4 Pin-tailed Whydahs (*Pin-tailed Whydah*), 2 Broad-tailed Paradise Whydahs (*V. obtusa*) and 1 Purple Indigobird (*V. purpurascens*). The estrildid recordings to match were 1 Bronze Mannikin, 1 Jameson's Firefinch, 1 Melba Finch, 2 Orange-winged Pytilias, 1 Zebra Waxbill, 3 Blue Waxbills and 2 Common Waxbills.

Participants were asked to match estrildid as well as *Vidua* recordings to act as a positive control. If participants were poor at matching *Vidua* recordings to their hosts, but also poor at matching estrildid calls to the calls of the same species, this could imply that the participant is not good at reading sonograms rather than that no mimicry is occurring. Prior to starting the experiment, an extrinsic quality filter of 50% was decided on. This meant that any participants that were unable to match more than 50% of estrildid begging calls to their own species would be excluded from the analysis. Participants had a range of prior experience with reading sonograms (from no experience to extensive experience). In the end, none of the 10 participants scored lower than 50% at matching estrildid sonograms, and so no participants were excluded.

If no mimicry was occurring, participants should match *Vidua* calls to the correct hosts with an accuracy of around 12.5% (1 in 8). If mimicry is occurring, participants should match *Vidua* begging calls to those of their host with an accuracy significantly greater than 12.5%. The observed versus expected percentages were compared using the prop.test function in R base stats package (R Development Core Team 2017).

Imperfect mimicry of begging calls

To test whether there are consistent differences in the begging calls of Pin-tailed Whydahs and their Common Waxbill hosts, linear mixed models were carried out to compare call parameters. Only the call types 4a and 4b are incorporated into the analysis as these were also the only call types investigated for mimicry. The begging calls of the other two Purple Indigobird and Broad-tailed Paradise Whydah were not compared to those of their hosts due to the small sample sizes available for these two parasite species.

Linear mixed models were implemented using the *lmer* function from the R package lme4 (Bates et al. 2015). To investigate whether call parameters varied significantly between species a model, in which species identity was included as a fixed effect and individual identity as a random effect was compared to one with no fixed effect. If the inclusion of the species identity as a fixed effect significantly improved the fit of the model, it was concluded that this call parameter differed significantly between the two species.

Postural mimicry

Chicks were filmed on a Canon Powershot SX50 HS Digital Camera while audio recordings were being made of their begging calls. This allowed the head movements of the chicks to be captured. Head movements of hosts and parasites were categorised into one of three types: 1) no movement; 2) left to right/ right to left movement and 3) clockwise /anticlockwise movement.

The comparisons made here are descriptive and subjective. More precise quantification of head movements was difficult because the exact position of the camera relative to the begging chick differed between recordings. Therefore, even if software tracking the bill tip was used to quantify head movements, the same movements recorded from different angles could be represented differently as movement through space. For now,

therefore, the decision was made to describe the head movements subjectively rather than to quantify them.

4.3 RESULTS

Visual mimicry

Visual mimicry between nestling parasites and hosts

Photographs of the mouth markings of three parasite-host pairs are shown in Figure 4.4 and Figure 4.5. These photographs clearly show that parasite mouths most closely match those of their specific host, compared to those of other hosts on those of any other estrildid finch species breeding sympatrically (Figure 4.6).

Nestling Pin-tailed Whydahs matched their Common Waxbill host in several features: the number of spots on the upper palate; the presence of white "arcs" on the outer gape flanges and black marks on the inner flanges; the presence of papillae on the lower gape edge; the two spots on the tongue. Similarly, nestling Purple Indigobirds matched their hosts Jameson's Firefinches in several features: the dark band on the upper bill tip; the number of spots on the upper palate; the structure of the gape flanges; the absence of marks on the tongue. Nestling Broad-tailed Paradise Whydahs matched the relatively plain mouth marking of their host Orange-winged Pytilia (Figures 4.4, 4.5 and 4.7). Interestingly, in the UV image, it became apparent that Orange-winged Pytilia have 3 dark spots on the upper palate which are not present in the human-visible spectrum. The single Broad-tailed Paradise Whydah UV image seems to also show a central dark spot, but the two lateral spots are not visible.

The PCA of the position of black markings across the upper palate of *Vidua* and estrildid finches also shows clustering between the mouth markings of *Vidua* parasites and their hosts (Figure 4.9). The clustering of parasite with host mouth markings is not as close as one might expect based on visually comparing the mouth markings in Figure 4.4. This discrepancy is likely due to the PCA focussing only on the distribution of black pattern on the inner palate and upper bill tip, and ignoring the gape flange structures and colour features. Additionally, PC1 and PC2 only capture a combined total of 33.1% of the variation in that subset of features.

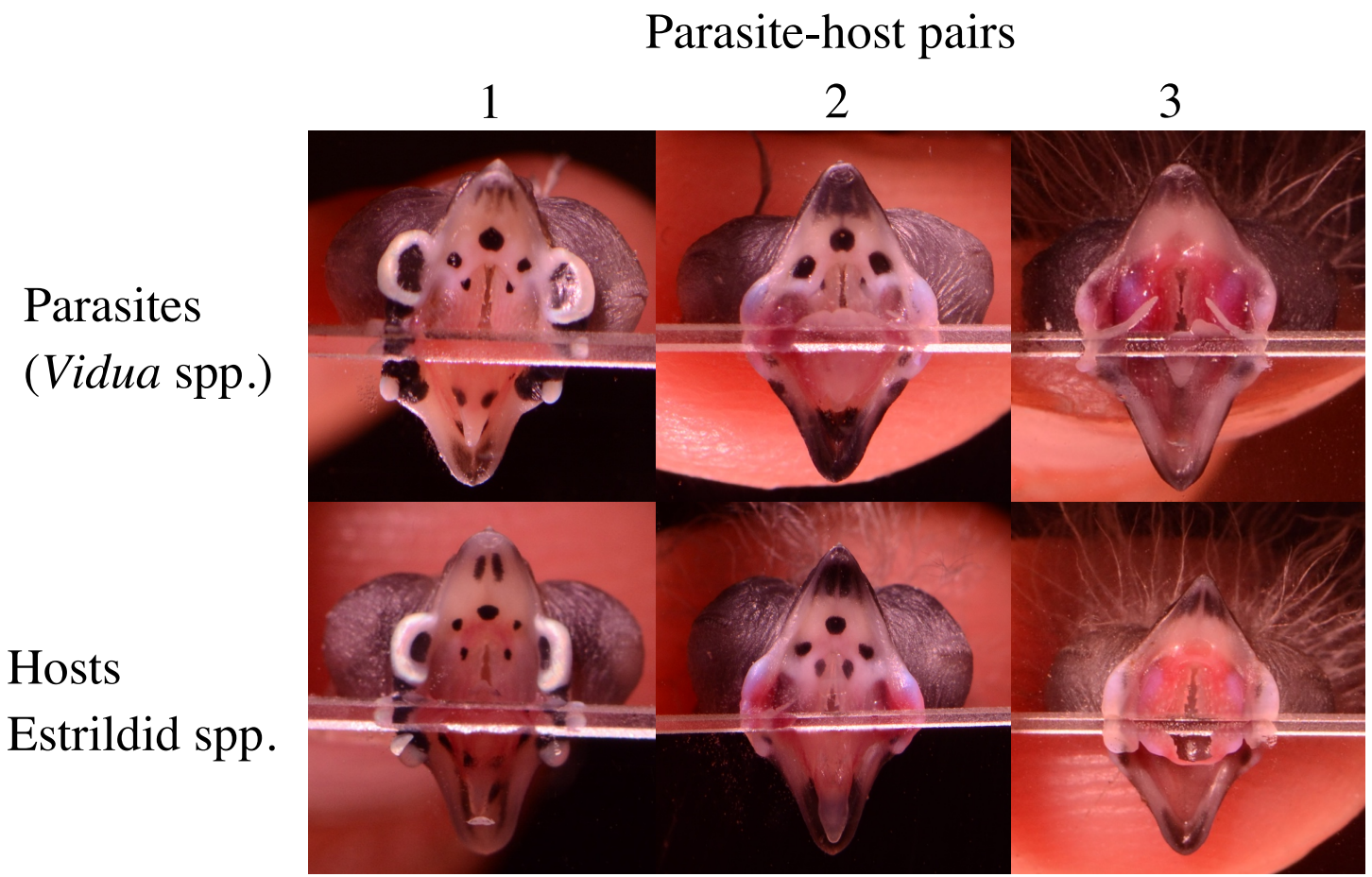


Figure 4.4. Mouth marking mimicry of their estrildid hosts by Vidua finches. Visible photographs of newly-hatched chicks, parasites (top row) and hosts (bottom row). Parasite-host pairs: 1) Pin-tailed Whydah (*V. macroura*) and Common Waxbill (*Common Waxbill*); 2) Purple Indigobird (*V. purpurascens*) and Jameson's Firefinch (*Lagonosticta rhodopareia*); 3) Broad-tailed Paradise Whydah (*V. obtusa*) and Orange-winged Pytilia (*Pytilia afra*).

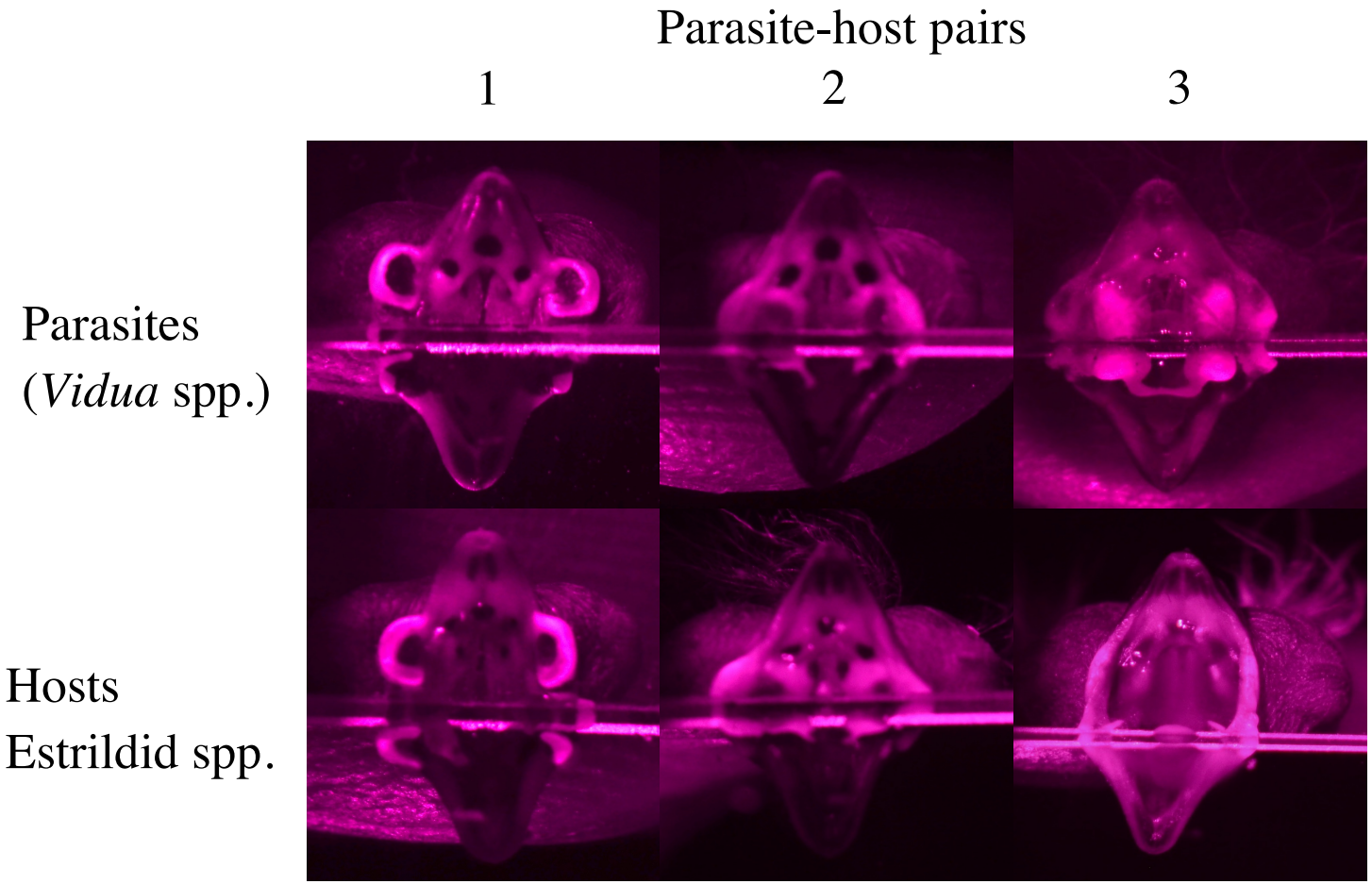


Figure 4.5. Ultra-violet photographs of newly-hatched chicks, parasites (top row) and hosts (bottom row). Parasite-host pairs: 1) Pin-tailed Whydah (V. macroura) and Common Waxbill (*Estrilda astrild*); 2) Purple Indigobird (*V. purpurascens*) and Jameson's Firefinch (*Lagonosticta rhodopareia*); 3) Broad-tailed Paradise Whydah (*V. obtusa*) and Orange-winged Pytilia (*Pytilia afra*). A brighter region in the photograph indicates more UV light reflected.

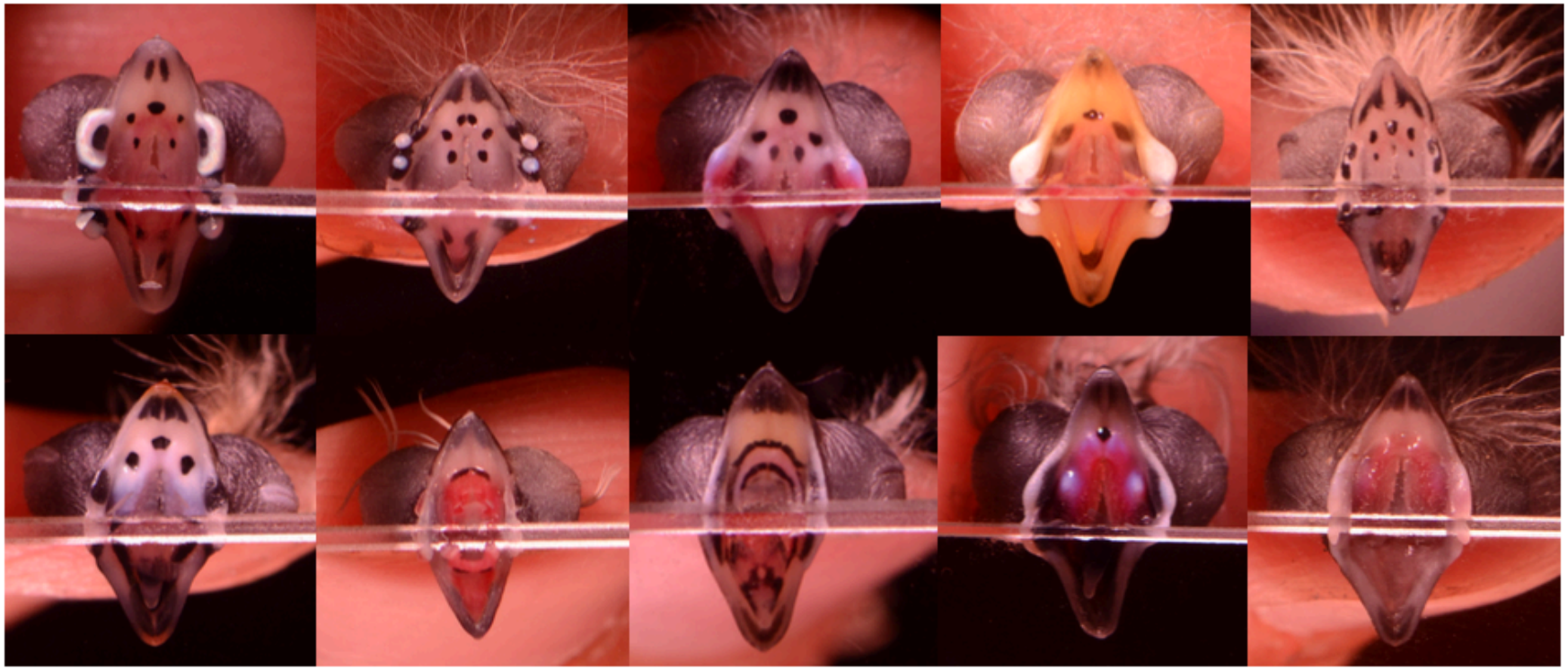


Figure 4.6. The diverse mouth markings of estrildid finches. Top row, left to right: Common Waxbill, African Quailfinch, Jameson's Firefinch, Red-billed Firefinch and Zebra Waxbill. Bottom row, left to right: Blue Waxbill, Locust Finch, Bronze Mannikin, Melba Finch, Orange-winged Pytilia. Images are arranged in order of decreasing ornamentation score. Common Waxbill and African Quailfinch have the highest ornamentation scores whilst Orange-winged Pytilia has the lowest. These are all the estrildid finch species present breeding at the study site in Zambia.

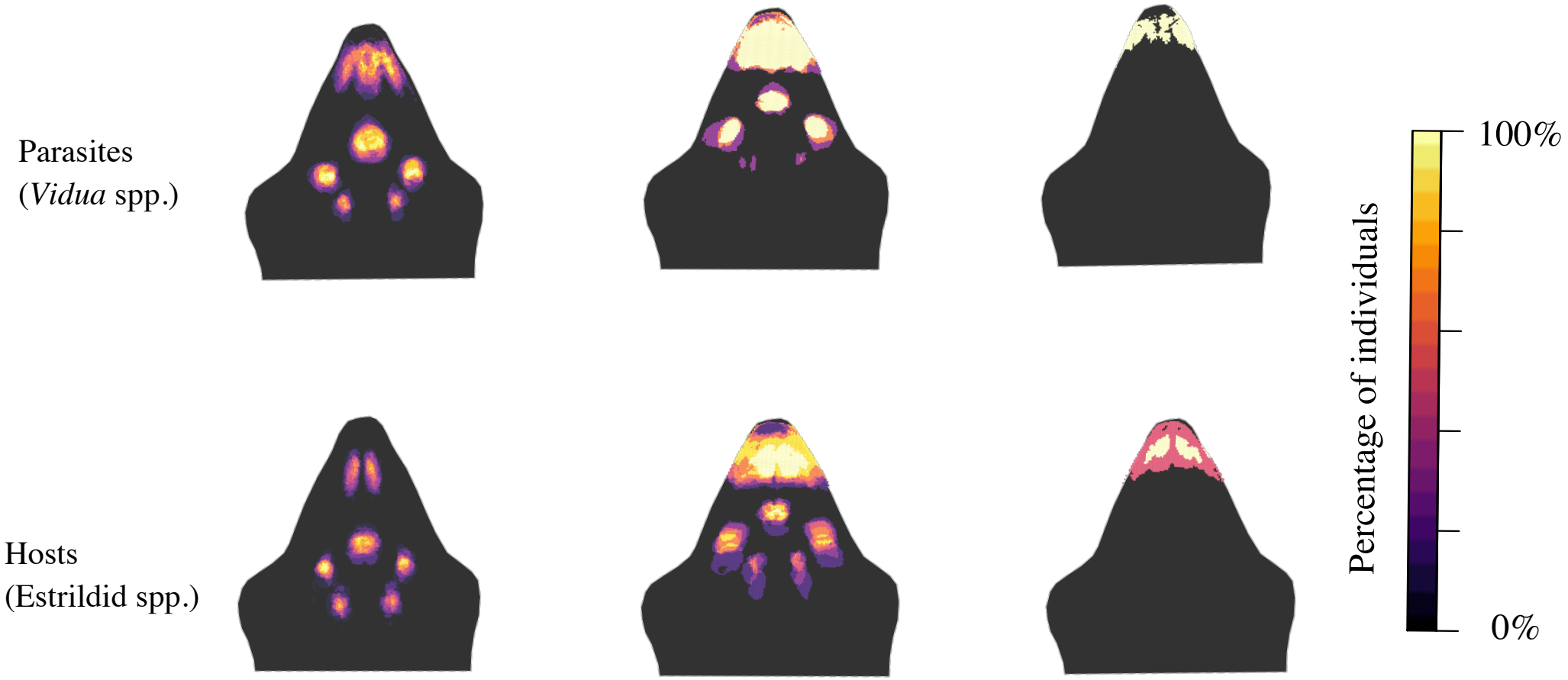


Figure 4.7. Composite images of the position of black patterns on the upper palate of Vidua finches and their estrildid hosts. The brighter the colour the greater the proportion of individuals exhibiting a black pattern in that position. Parasite-host pairs: 1) Pin-tailed Whydah (n = 9) – Common Waxbill (n = 11); 2) Purple Indigobird (n = 3) – Jameson's Firefinch (n = 6); 3) Broad-tailed Paradise Whydah (n = 1) – Orange-winged Pytilia (n = 2).

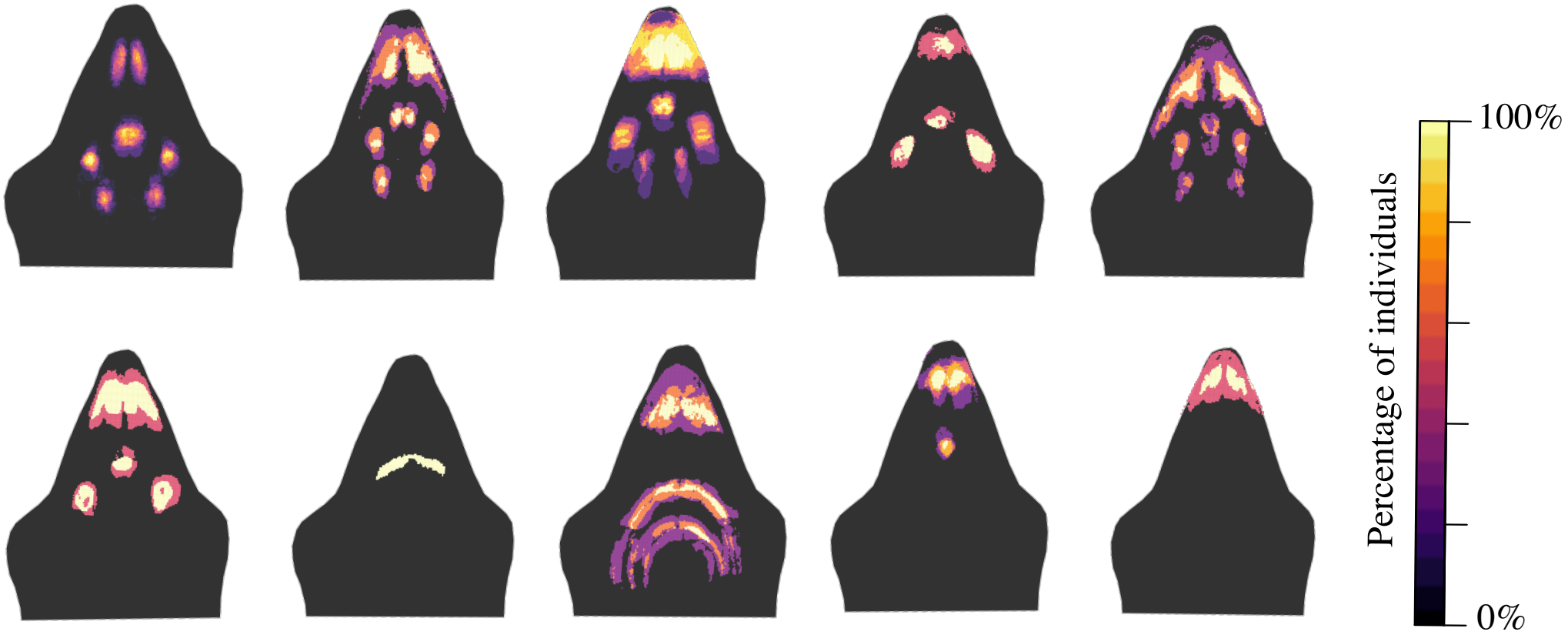


Figure 4.8. Composite images of the pattern of black markings on the upper palate of nestling estrildid finch species occurring sympatrically at our field site in southern Zambia. The brighter the colour the greater proportion of individuals of that species exhibiting a black pattern in that position. Top row, left to right: Common Waxbill (n = 17), African Quailfinch (n = 3), Jameson's Firefinch (n = 8), Red-billed Firefinch (n = 5), Zebra Waxbill (n = 3). Bottom row, left to right: Blue Waxbill (n = 2), Locust Finch (n = 1), Bronze Mannikin (n = 3), Melba Finch (n = 5), Orange-winged Pytilia (n = 2).

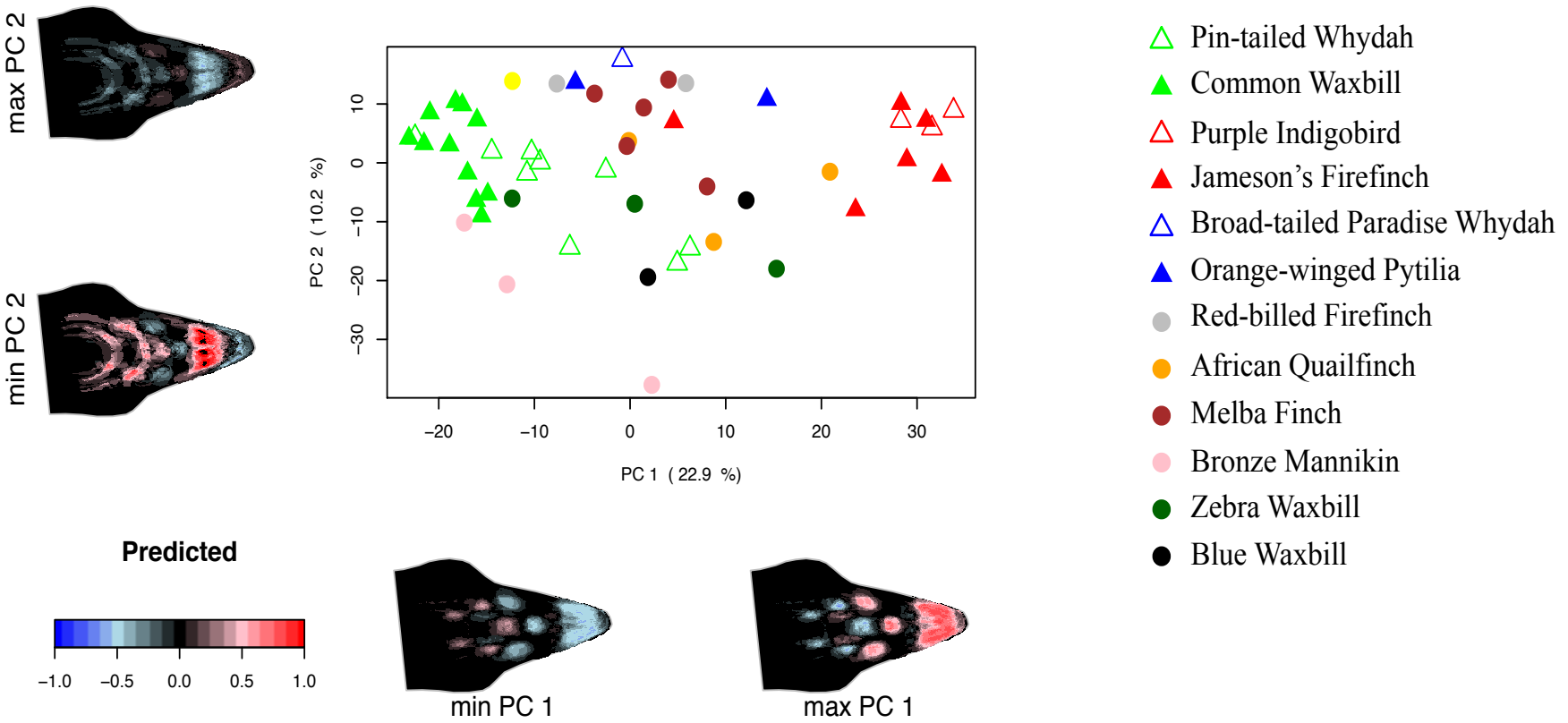


Figure 4.9. Principal Component Analysis of the pattern of black markings on the upper palate of Vidua and estrildid finches. The x-axis shows principal component 1 (PC1) which explains 22.9% of the pattern variation among the species. The y-axis shows principal component 2 which explains a further 10.2% of the variation. Three parasite-host pairs are shown in the diagram: 1) Pin-tailed Whydah and Common Waxbill; 2) Purple Indigobird and Jameson's Firefinch; 3) Broad-tailed Paradise Whydah and Orange-winged Pytilia. Parasite individuals are represented by open triangle symbols. Their respective hosts are represented by closed triangle symbols of the same colour as their parasite. Other sympatric estrildids are represented by closed circles of different colours. Diagram generated by Steven van Bellegham using the R package patternize (Bellegham et al. 2017).

Differences between parasite and host mouth markings

Upper palate spot size

Newly-hatched Pin-tailed Whydah nestlings (n = 9) had significantly larger upper palate spots than newly-hatched Common Waxbill nestlings (n = 11) (Figure 4.10). This was true when the 5 upper palate spots were considered together (Kruskal-Wallis, chi-squared = 6.48, $p < 0.05$) and when focussing on the distal three spots individually: spot 1 (chi-squared = 9.94, $p < 0.01$), spot 2 (chi-squared = 7.70, $p < 0.01$) and spot 5 (chi-squared = 10.44, $p < 0.01$) were all significantly larger in Pin-tailed Whydah nestlings compared to Common Waxbill nestlings. By contrast, for the proximal two spots (spots 3 and 4), there was no significant difference in size (spot 3: chi-squared = 0.175, $p > 0.6$; spot 4: chi-squared = 0.578, $p > 0.4$). See Figure 4.10 for the position of each spot on the mouth. The larger relative size of the front 3 spots in Pin-tailed Whydahs compared to Common Waxbills is visible on the composite images in Figure 4.7 and in Figure 4.12.

The upper palate spot sizes of Purple Indigobird (n = 3) and its host, Jameson's Firefinch (n = 6), were also compared (Figure 4.11). There was no significant difference in overall spot size when the five upper palate spots were combined (chi-squared = 0.0667, $p > 0.7$). There is a trend towards Purple Indigobirds having spot 1 larger than Jameson's Firefinch (chi-squared = 3.27, $p = 0.0707$). The two lateral palate spots (spot 2 and 5) showed no evidence for size differences between Purple Indigobirds and Jameson's Firefinch (spot 2: chi-squared = 0.420, $p > 0.5$; spot 5: chi-squared = 0.600, $p > 0.4$). However, there is a suggestion that the two mediolateral spots (3 and 4) are smaller in Purple Indigobird compared to Jameson's Firefinch. Spot 3 is significantly smaller (chi-squared = 4.30, $p < 0.05$), whereas spot 4 is not significantly smaller (chi-squared = 1.68, $p > 0.1$). However, the boxplot in figure 4.11 suggests that, with larger sample sizes, the latter difference might be significant.

Broad-tailed Paradise Whydah and its host Orange-winged Pytilia lack upper palate spots, at least in the visible spectrum, so spot sizes were not compared.

Bill tip pattern

The tip of the upper mandibles of Pin-tailed Whydah and Common Waxbill show a marked difference (Figure 4.4, 4.7 and 4.12). Common Waxbills generally have two straight black

lines, whereas Pin-tailed Whydahs have an "m" shape (Figure 4.12). This is the most obvious qualitative difference in the mouth markings of the two species.

By contrast, the bill tip patterns of Purple Indigobird and Broad-tailed Paradise Whydah are solid wedges of black which broadly match those found in their respective hosts (Figure 4.7).

Luminance of outer gape flanges

There were no significant differences in the level of stimulation of the double cone channel by the white outer gape flanges of Pin-tailed Whydah (n = 5) versus Common Waxbill nestlings (n = 5) (chi-squared = 0.535, $p > 0.4$).

Luminance contrast between white outer and black inner gape flanges

Luminance contrast between the white outer and black inner gape flanges for each chick was measured using Just Noticeable Differences (JNDs). Internal contrast did not differ for Common Waxbill and Pin-tailed Whydah for the chick's right-hand flange (chi-squared = 0.273, $p > 0.6$). However, JNDs were significantly higher for Common Waxbills compared to Pin-tailed Whydahs when focussing on the chick's left-hand gape (chi-squared = 6, $p < 0.05$). However, when examining through the mouth marking images used in the colour and luminance analysis, the 5 Pin-tailed Whydah individuals show strong spectral reflectance on the left-hand side interior gape. This reflectance is either absent or very slight in the 5 Common Waxbill individuals. Therefore, the degree of internal contrast shown by the chick's right-hand gape is a more accurate measure than that on the left, as the right-hand side does not show this spectral reflectance. The reason for this discrepancy is due that the camera flash was always located on the chick's left-side, meaning that it was exposed to the flash more than the right-hand side.

Colour contrast between black palate spots and background palate

Internal contrast in colour between spots and the background palate was quantified using JNDs between each of the 5 spots and adjacent areas of the palate. There was no significant difference between Pin-tailed Whydah and Common Waxbill in contrast between spots and adjacent palate areas for any of the 5 spots (chi-squared < 0.884, $p > 0.3$ for all comparisons).

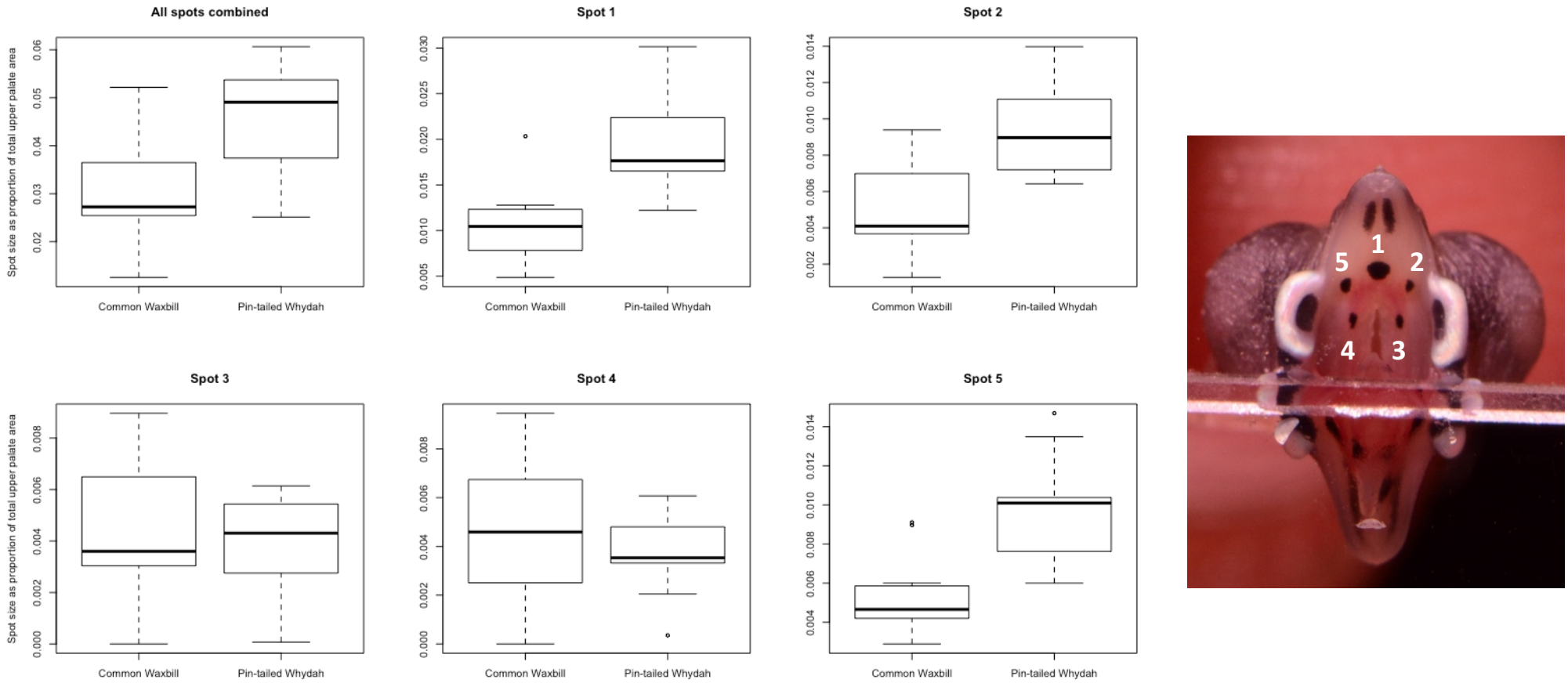
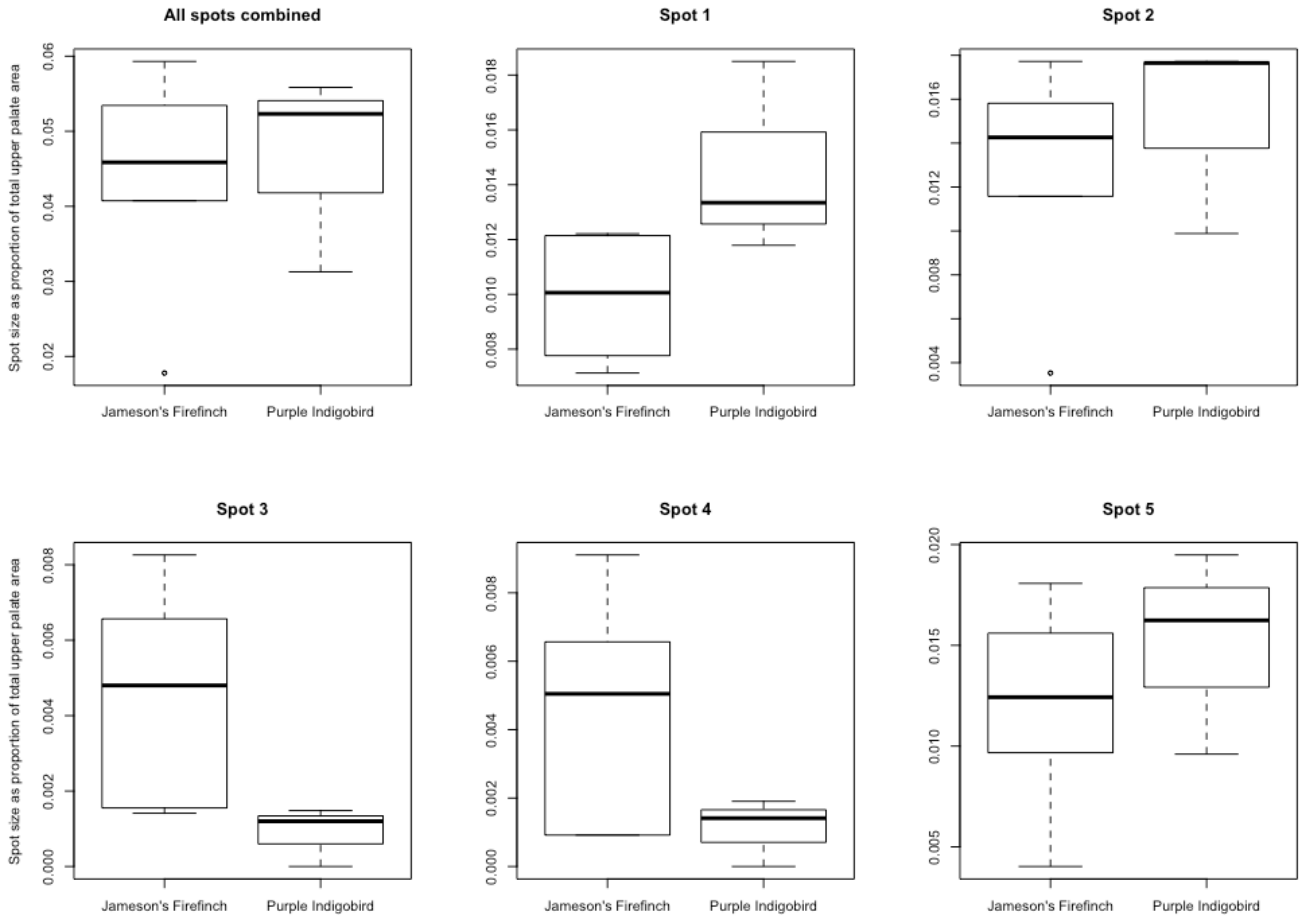


Figure 4.10. Comparing the size of black spots in the centre of the upper palate between Pin-tailed Whydah (Pin-tailed Whydah) (n = 9) and Common Waxbill (Common Waxbill) (n = 11). Spot size is given as a proportion of the total upper palate area. Spot numbers are shown in the image on the right. Spot 1 is the medial palate spot, spot 2 is the lateral palate spot (left), spot 3 is mediolateral palate spot (left), spot 4 is mediolateral palate spot (right), spot 5 is lateral palate spot (right). Spots 1, 2 and 5 are significantly larger in Pin-tailed Whydah nestlings compared to Common Waxbill nestlings. There are no significant differences in the sizes of spots 3 and 4.



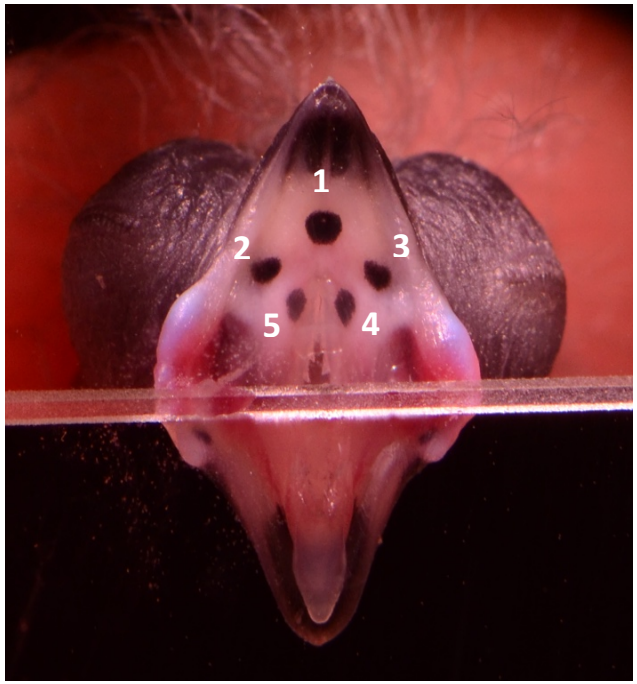


Figure 4.11. Comparing the size of black spots in the centre of the upper palate between Purple Indigobird (n = 3) and Jameson's Firefinch (n = 6). Spot size is given as a proportion of the total upper palate area. Spot numbers are shown in the image on the right. Spot 1 is the medial palate spot, spot 2 is the lateral palate spot (left), spot 3 is mediolateral palate spot (left), spot 4 is mediolateral palate spot (right), spot 5 is lateral palate spot (right). There is a significant difference in the size of spot 3 and a trend for a larger spot 1 in Purple Indigobird. The other comparisons are non-significant.

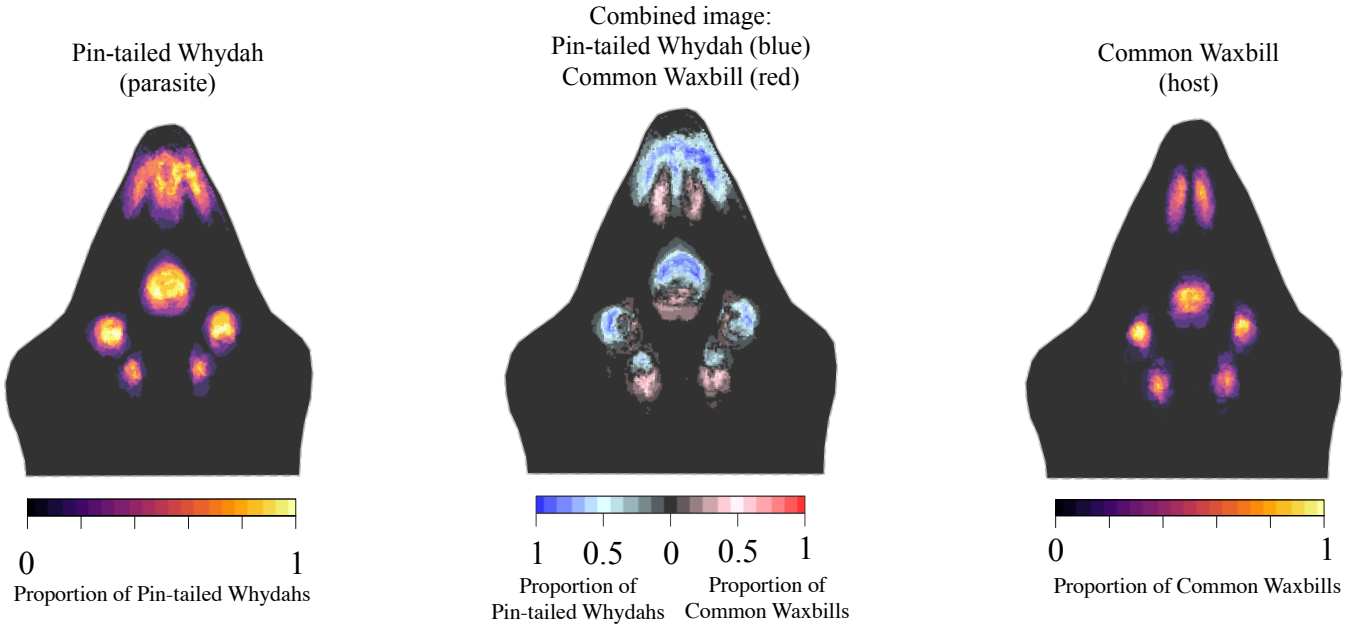


Figure 4.12. Comparing pattern of black markings on the mouths of newly-hatched Pin-tailed Whydah (leftmost image) and Common Waxbill (rightmost image) nestlings. The central image shows the patterns of both species overlaid. Blue colours represent areas where Pin-tailed Whydah nestlings have markings, red areas where Common Waxbills have markings. Points with no overlap in red and the blue indicate regions of consistent difference between the mouth markings of Pin-tailed Whydahs and Common Waxbills. Key areas of difference are the pattern of black on the bill tip and the sizes of the distal 3 palate spots. Images are formed as composites of photographs from multiple individuals (Pin-tailed Whydah, n = 9; Common Waxbill, n = 11).

Vocal mimicry

Begging call mimicry between* Vidua *and their hosts

First, a multinomial logistic regression model (MLR) was used to generate a classification function using estrildid finch begging call data. *Vidua* begging calls were subsequently entered into the model. The accuracy of the classifications are shown in table 4.1. For the 5 Pin-tailed Whydah individuals entered into the model, I will focus first on those producing call type 4a (the first part of the two-note "we-chee" call, see figure 4.13). The mean percentage of calls correctly assigned to the right host species by the model was 76%, and the median was 100%. For Pin-tailed Whydahs producing call note 4b (the second part of the "we-chee" call, see figure 4.13), the mean percentage of calls correctly assigned to the right host species by the model was 64%, and the median was 70%. If parasite calls were being assigned randomly amongst the 9 estrildid states (8 species in total, but with call type 4a and 4b assigned separate categories in the model, making 9 total), the expected proportion correct would be 2 in 9 or 22.2%.

The two Broad-tailed Paradise Whydah calls were assigned to the correct host species, Orange-winged Pytilia, with a mean level of accuracy of 45%. For Broad-tailed Paradise Whydah individual 1, the 2/10 incorrectly assigned call notes were assigned to Melba Finch rather than to Orange-winged Pytilia. These two estrildid species are in the same genus and have similar calls. Therefore, this individual was assigned to the correct host genus with 100% accuracy by the model.

Second, discriminant function analysis (DFA) was used to generate a classification function using estrildid finch begging call data, and *Vidua* begging calls again entered into this model. The results were broadly in support of those from the multinomial regression approach (see Table 4.1). DFA did slightly better than MLR in assigning Pin-tailed Whydah calls to the correct host species, but slightly worse in assigning Broad-tailed Paradise Whydah and Purple Indigobirds to their respective hosts. LD1 explained 54.9% of the variance in host begging calls, and LD2 explained a further 28.1%.

A visualisation of the spread of host and parasite calls on linear discriminant 1 and 2 is shown in Figure 4.17.

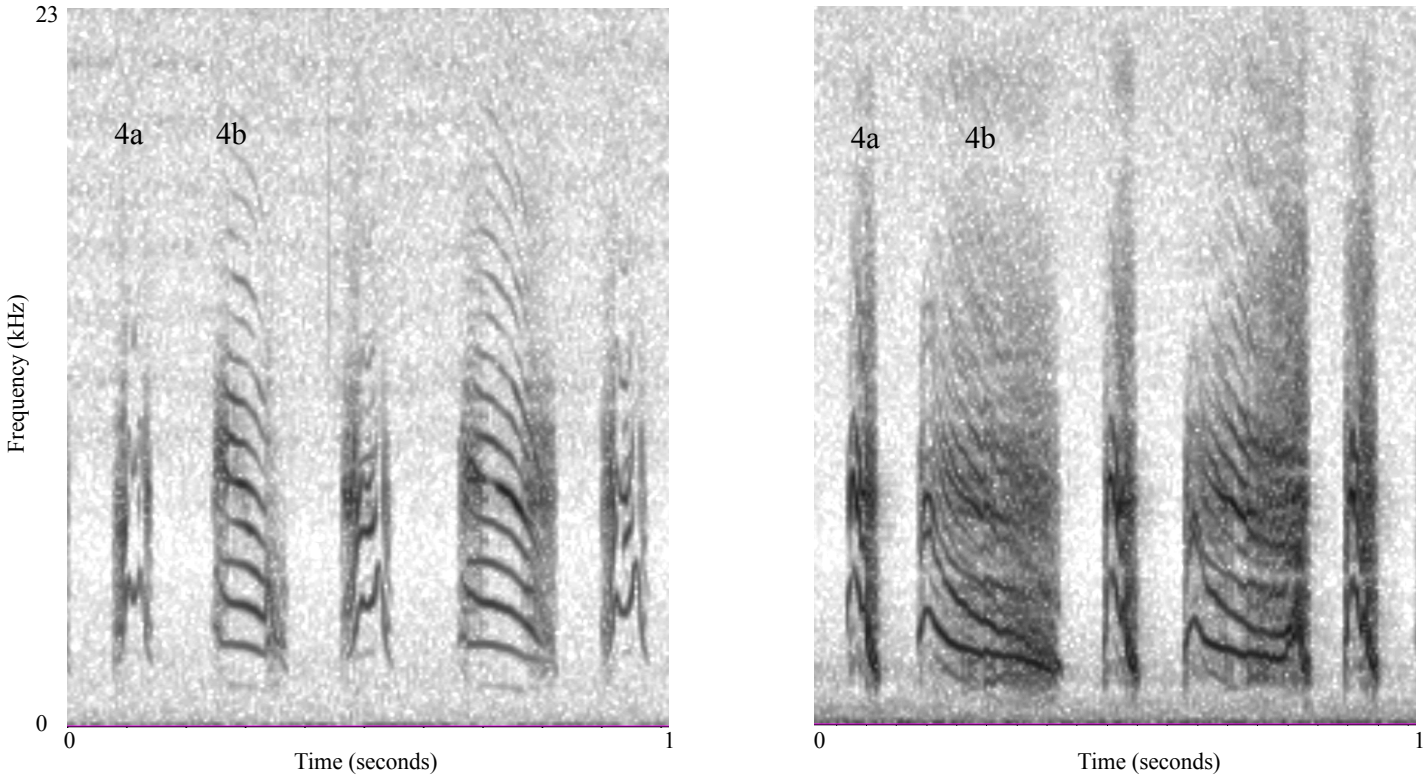


Figure 4.13. One second sections of begging from a nestling Common Waxbill (left) and its parasite Pin-tailed Whydah (right). Both sonograms show examples of type 4 begging calls. This is a two-note call transcribed as "we-chee". The first note (4a) is short and the second note is longer (4b). The two call sub-types are given in rapid alternation. Call type 4b of Pin-tailed Whydah usually lasts longer than call type 4b of Common Waxbill. No other estrildid or Vidua finch gives this call type (see Figure 4.12-14). This suggests that Pin-tailed Whydahs are mimicking this begging call type of their Common Waxbill host. Common Waxbill chick in this figure had primaries about one quarter irrupted from pin. The Pin-tailed Whydah chick had primaries nearly fully irrupted from pin.

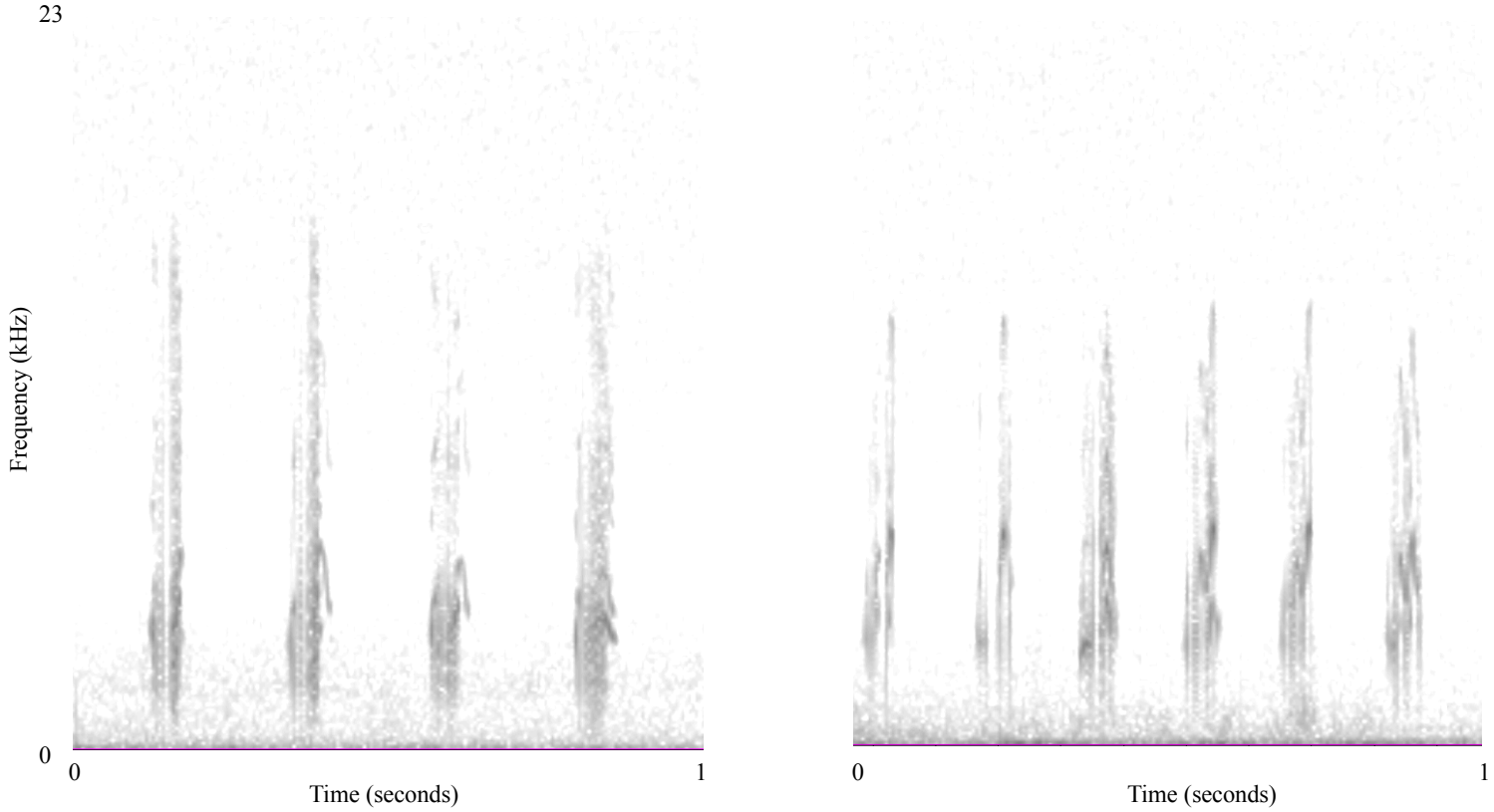


Figure 4.14. One second sections of begging from a nestling Jameson's Firefinch (left) and its parasite, Purple Indigobird (right). Both individuals in this recording had their primaries irrupted from pin and were therefore at mid to late developmental stage.

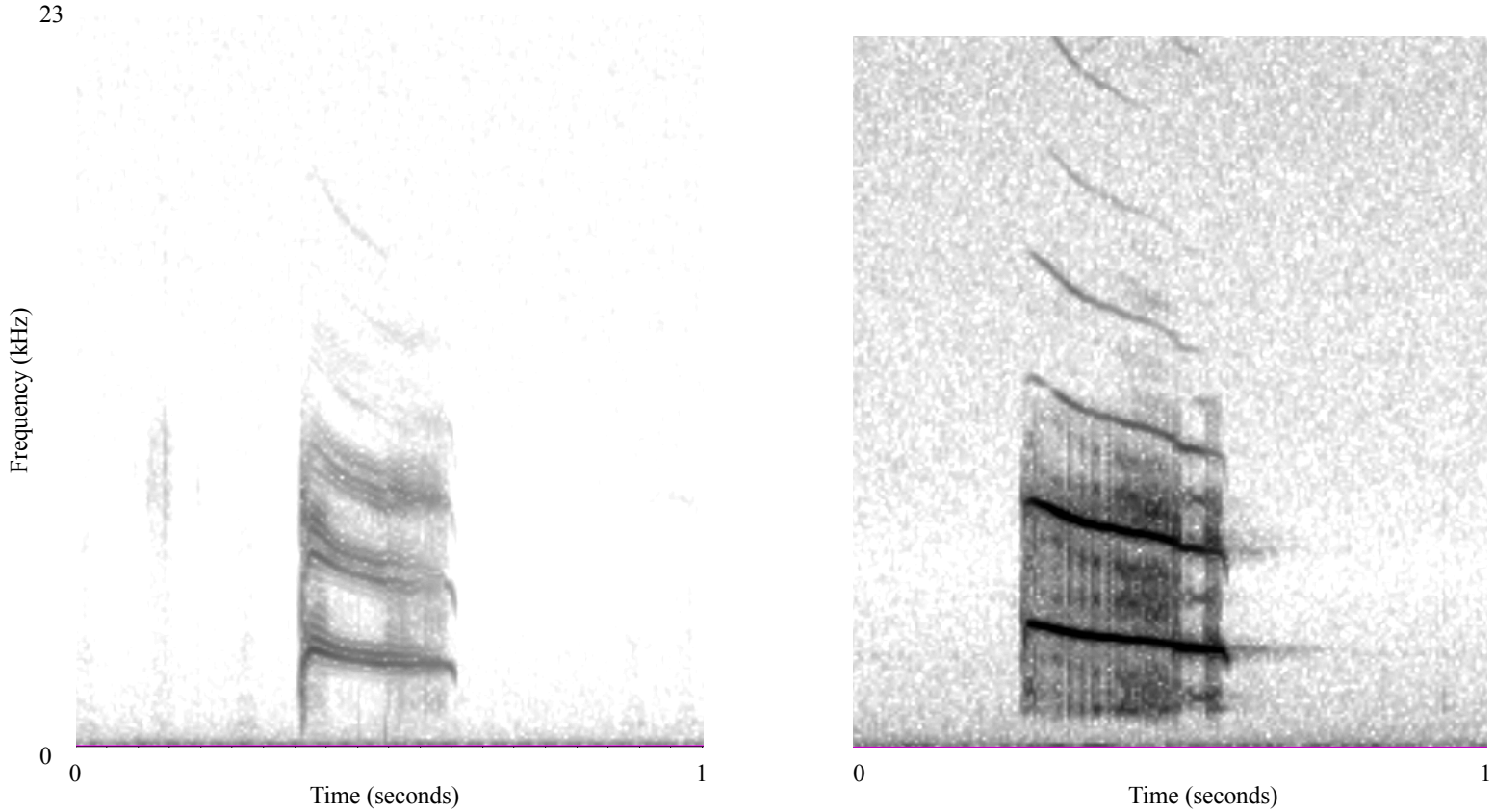


Figure 4.15. One second sections of begging from a nestling Orange-winged Pytilia (left) and its parasite Broad-tailed Paradise Whydah (right). Both chicks in these recordings had their primaries irrupted from pin and so were in mid to late-development

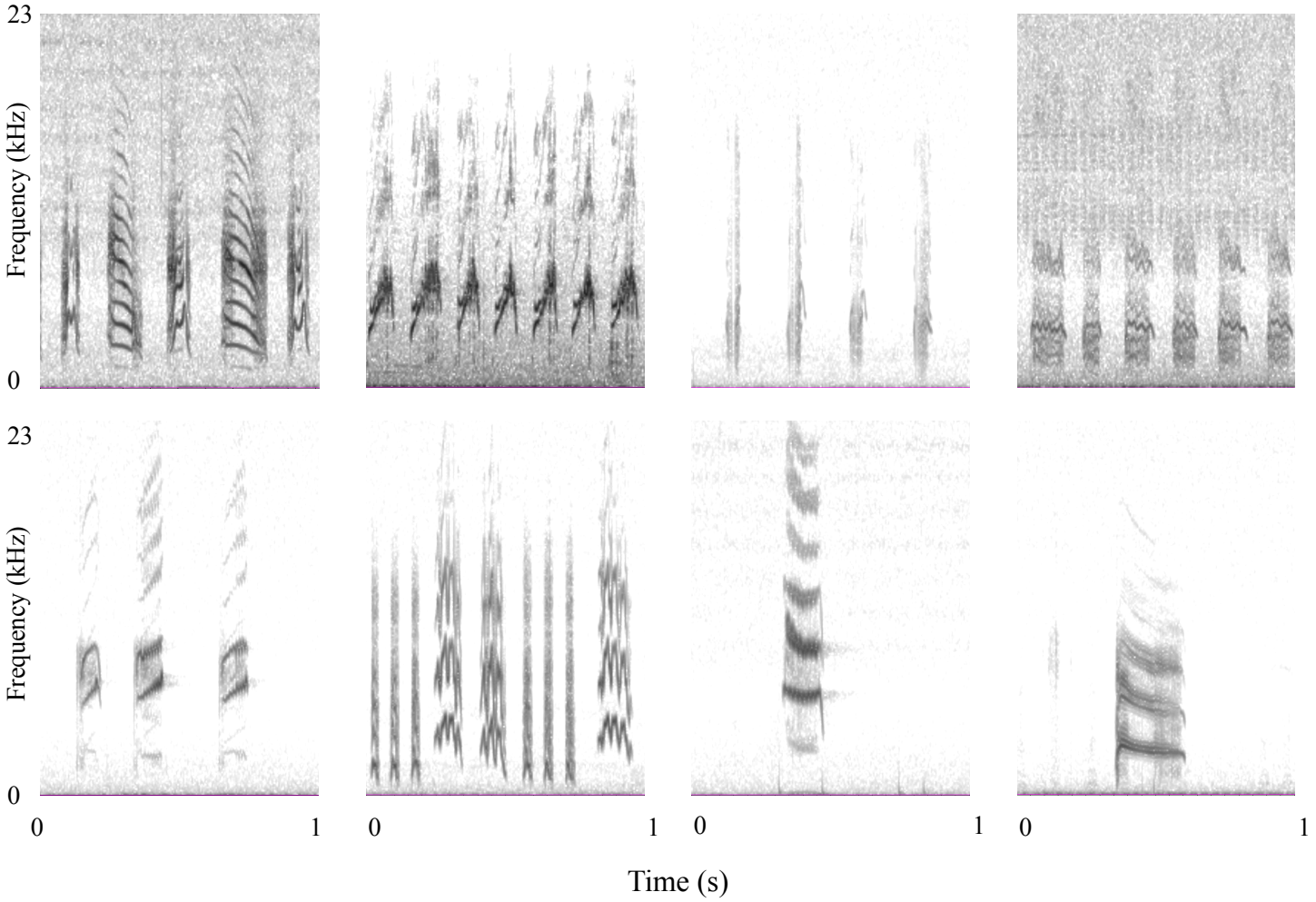


Figure 4.16. The diverse begging calls of nestling estrildid finches. These are all one second sections of begging from chicks in mid to late development (primaries have already irrupted from pin). Top row, left to right: Common Waxbill, African Quailfinch, Jameson's Firefinch, Zebra Waxbill. Bottom row, left to right: Blue Waxbill, Bronze Mannikin, Melba Finch, Orange-winged Pytilia.

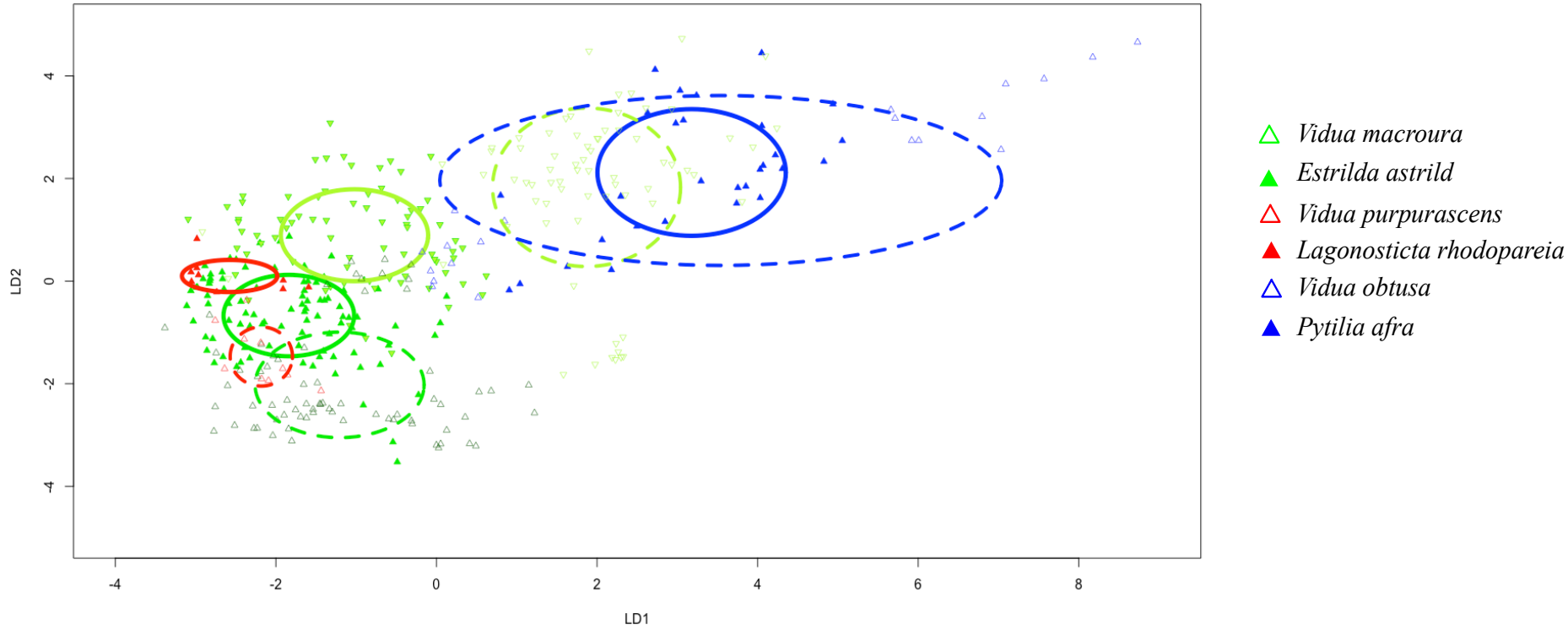


Figure 4.17. Linear discriminant analysis of the begging calls of three estrildid host species and their respective Vidua parasites. The linear discriminant function was generated by inputting begging calls from all 7 sympatric estrildid species. The Vidua values were calculated by being entered into this formula. Each point on the graph represents an individual call note. Ellipses show are centred on the mean LD1 and LD2 values for a given species and have a width of 2 times the standard deviation in LD1 and and height of 2 times the standard deviation in LD2. Solid ellipses are for host species. Broken ellipses are for Vidua parasite species. Parasite-host pairs are in the same colour. Dark green is call type 4a, light green is call type 4b for both *Estrilda astrild* and *Vidua macroura*.

Table 4.1. Classification of *Vidua* begging calls predicted by multinomial logistic regression (MLR) and discriminant function analysis (DFA) trained only on the begging calls of the eight locally occurring estrildid finch species. The model was trained on two call types for Common Waxbill (*E. astrild*), the host of Pin-tailed Whydah (*V. macroura*). Therefore, for *V. macroura* assignments, the first column states the percentage of sonograms matching to *E. astrild* (either call type 4a or 4b), and the second states the percentage matching to the corresponding call type of *E. astrild* specificially (rather than just to *E. astrild* of either call type). If random, the expected percentage of matches to the correct species would be 22.2% (2/9), and the expected percentage match to the correct call type would be 11.1% (1/9). Observed values that were significantly greater (at 5% level) than expected by chance are in bold.

***Vidua* species**	**Individual**	**Call type produced**	**Percentage of calls assigned to correct host *species* by model**		**Percentage of calls assigned to correct host *call type* by model**	
			MLR	**DFA**	**MLR**	**DFA**
Pin-tailed Whydah	1	4a	**80**	**100**	**80**	**100**
	2	4a	**100**	**100**	**100**	**100**
	3	4a	**100**	**100**	0	**80**
	4	4a	**100**	**100**	**100**	**100**
	5	4a	0	0	0	0
	1	4b	**100**	**80**	**100**	**80**
	2	4b	**80**	40	**80**	**40**
	3	4b	**70**	30	**70**	30
	4	4b	**70**	**80**	**70**	**80**
	5	4b	0	0	0	0
Broad-tailed Paradise Whydah	1	NA	**80**	**70**	NA	NA
	2	NA	10	0	NA	NA
Purple Indigobird	1	NA	**50**	20	NA	NA

Human assessment of begging call mimicry by Vidua

Human participants could correctly match the sonograms of *Vidua* nestling begging calls to their specific hosts with a high level of accuracy (Table 4.2). Across the 3 *Vidua* species, the mean percentage of sonograms correctly matched to their host was 72.9%. 82.5% of *Pin-tailed Whydah* begging calls were correctly assigned to their host Common Waxbill.

It should be noted that all the *V. obtusa* sonograms that were not matched to the correct host (*Pytilia afra*) were instead matched to Melba Finch (*P. melba*), a closely related congener with a similar begging call (see Figure 4.14 to compare sonograms). Human assignments of *V. obtusa* were therefore 100% accurate at the host genus level, but only 50% accurate at the host species level. Only a single *V. purpurascens* recording was available for participants to match. 8 of the 10 participants matched it to its correct host, *Lagonosticta rhodopareia*. The proportion of correct matches for each participant for Purple Indigobird and Broad-tailed Paradise Whydah were not significantly different from the null expectation (12.5%) due to the small number of sonograms available for these species (one and two respectively) to assign to hosts.

***Begging call differences between* Pin-tailed Whydah *and its host* Common Waxbill**

The only significant difference between the calls of *V. macroura* and its host, *E. astrild*, was in the duration of the type 4b call note (Table 4.3). *V. macroura* has significantly longer type 4b begging calls than does *E. astrild* (linear mixed model, chi-squared = 11.6, $p < 0.001$). This difference is visible in the sonograms in Figure 4.13.

Table 4.2. Human assessment of similarity between the sonograms of Vidua and their hosts. 10 participants were asked to match sonograms to one of 8 potential "host" sonograms (see methods for details). Note that all the V. obtusa mismatches were to Pytila melba, a species in the same genus as the true host, P. afra, and with similar begging calls. The expected percentage correct if parasite sonograms were being matched randomly to host's by participants would be 12.5% (1 in 8). Percentages significantly greater than 1 in 8 are highlighted in bold.

Participant	**% of sonograms matched to correct species**				
	Vidua	**estrildids**	***V. macroura***	***V. obtusa***	***V. purpurascens***
1	**57.1**	**72.7**	**75.0**	50.0	0.0
2	**71.4**	**72.7**	**75.0**	50.0	100.0
3	**71.4**	**72.7**	**75.0**	50.0	100.0
4	**85.7**	**63.6**	**100.0**	50.0	100.0
5	**71.4**	**72.7**	**75.0**	50.0	100.0
6	**71.4**	**63.6**	**75.0**	50.0	100.0
7	**71.4**	**72.7**	**75.0**	**100.0**	0.0
8	**85.7**	**90.9**	**100.0**	50.0	100.0
9	**85.7**	**81.8**	**100.0**	50.0	100.0
10	**57.1**	**63.6**	**75.0**	0.0	100.0
Mean	72.9	72.7	82.5	50	80

Postural mimicry between* Vidua *and their hosts

Of the 8 species of estrildid finch occurring at the field site in southern Zambia, Common Waxbill was the only species not to move its head whilst begging. Correspondingly, its parasite, Pin-tailed Whydah, also never moved its head whilst begging. The only detectable difference in the begging display between newly-hatched Pin-tailed Whydahs and newly-hatched Common Waxbills was a wing-waving movement carried out by Pin-tailed Whydahs, which Common Waxbills were not observed to do. Pin-tailed Whydahs waved only one wing at a time, the wing being waved being the one on the side of the body that the open mouth was pointing towards. Pin-tailed Whydahs usually have a few wispy hair-like feathers on their wings which Common Waxbill chicks lack. Interestingly, a single Pin-tailed Whydah individual was observed to lack these wing feathers but still made the waving movement.

The absence of head movements in the Common Waxbill – Pin-tailed Whydah host parasite pair contrasts with the other estrildid finches and their *Vidua* parasites. Broad-tailed Paradise Whydah and its host, Orange-winged Pytilia both moved their heads left-to-right when begging. A similar left-to-right movement was also seen in a newly-hatched Purple Indigobird chick and its host the Jameson's Firefinch.

Table 4.3. Comparing call parameters of call types 4a and 4b between Pin-tailed Whydah and Common Waxbill. For each parameter, log-likelihoods of two linear mixed models (with and without species identity as a fixed effect) were compared with an F test with 1 degree of freedom. Chi-squared and p-values from the F test are included in each cell of the table. Chick individual identity was included as a random effect in all models. 16 comparisons were made in this table. Therefore, the significance level threshold was shifted accordingly from 0.05 to 0.003125 to correct for multiple testing. The only significant difference is the longer call duration of type 4b calls, compared to Common Waxbill type 4b calls.

Call parameter	**Call Type**	
	4a	**4b**
Call duration	0.338, $p > 0.5$	**11.6, $p < 0.001$**
Frequency bandwidth	1.36, $p > 0.2$	1.05, $p > 0.3$
Peak frequency	2.60, $p > 0.1$	0.0292, $p > 0.8$
Centre frequency	1.40, $p > 0.2$	0.0175, $p > 0.8$
Minimum frequency	0.210, $p > 0.6$	0.362, $p > 0.5$
Maximum frequency	1.68, $p > 0.1$	1.22, $p > 0.2$
Energy	0.0476, $p > 0.8$	0.0802, $p > 0.7$
Aggregate entropy	0.074, $p > 0.7$	0.0309, $p > 0.8$

4.4 DISCUSSION

In this chapter, I provide the first quantitative evidence for begging call and mouth marking mimicry between three pairs of brood-parasitic *Vidua* finches and their respective hosts: Pin-tailed Whydah and its host Common Waxbill, Purple Indigobird and its host Jameson's Firefinch, and Broad-tailed Paradise Whydah and its host Orange-winged Pytilia. I also provide a descriptive account of postural movements of parasites and hosts during begging, which suggest that these too may be mimetic. I show that, for the Pin-tailed Whydah – Common Waxbill pairing, there are consistent differences in the mouth markings, begging calls and postural displays between parasite and host. This is the first time that evidence has been presented for imperfect mimicry of hosts by a *Vidua* species. I also place the begging calls and mouth markings of the 3 parasite-host pairs in a broader context of the begging calls and mouth markings of non-host sympatric estrildid species. This provides further evidence that *Vidua* begging displays match those of their specific hosts more closely than those of any other co-occurring estrildid finch.

Visual mimicry

The visual match between the three *Vidua* species and their respective hosts in mouth markings is shown to be very precise (Figure 4.4), and to match the pattern and colours of their host markings far more closely than any other co-occurring estrildid finch species (Figure 4.6). This mimicry appears to extend into the UV spectrum too (Figure 4.5).

However, despite *Vidua* nestlings appearing to match the mouth markings of their host more closely than the markings of any other sympatric estrildid, there are consistent differences in mouth markings in at least one of the host-parasite pairs. The distal three upper palate spots of Pin-tailed Whydah nestlings were larger than the corresponding spots of Common Waxbill nestlings. Here spot size was measured in proportion to the total area of the mouth, and so any size discrepancies are not just due to Pin-tailed Whydahs being slightly larger overall than Common Waxbills at hatching. Interestingly, it is only the distal three spots that are larger, whereas the proximal two spots are the same size. Additionally, the pattern of black on the upper

palate near the bill tip is completely different in Pin-tailed Whydah and Common Waxbills (Figures 4.4 and 4.7).

Returning to the three hypotheses put forward in the introduction for the existence of imperfect mimicry, discrepancies between parasite and host traits could be due to: 1) weak selection from hosts; 2) genetic/developmental constraints in the parasites; 3) selection for parasite signals that are 'improved' versions of host signals and so better at manipulating parents (i.e. superstimuli); 4) evolutionary lag. I will now consider each hypothesis in turn.

1) Weak selection

Estrildid host parents are known to discriminate against non-mimetic chicks (Payne and Payne 2002; Payne et al. 2001; Schuetz 2005b, Chapter 5 of this thesis). The discrimination against odd-looking chicks can be fine-scale. For example, Schuetz (2005) coloured in black the white gape flanges of Common Waxbill chicks and showed that these individuals grew less well than sham-manipulated waxbill chicks. However, there was no difference in survival between waxbill chicks with black gape flanges and those with white, so it is not clear whether this manipulation would have any long-term fitness consequences. By contrast, my experiments (Chapter 5) and previous work (Payne et al. 2001) shows that chicks of different species with very different appearances do survive worse in foreign estrildid nests than do estrildid nestlings raised by a parent of the same species. Therefore, it is possible that estrildid finch parents select for nestlings with broadly similar mouth marking appearance, but that very fine-scale differences such as exact spot size and bill tip pattern do not influence provisioning rate.

2) Genetic/developmental constraint

In the context of *Vidua* mouth markings, the discrepancy in spot size is unlikely to be due to genetic/developmental constraint. This is because different *Vidua* species have evolved a wide diversity of mouth markings depending on which host they are mimicking. This includes reducing the sizes of certain palate spots to being completely absent in some *Vidua* species. For example, Village Indigobirds parasitizing Red-billed Firefinches have only 3 upper palate spots, with the two mediolateral spots lost entirely (Payne 2005b, and see Figure 4.6). Additionally,

Broad-tailed Paradise Whydahs parasitizing Orange-winged Pytilias have lost palate spots entirely (although there are some indications that dark spots are still present in the UV spectrum – see Figure 4.5). Therefore, it is likely that *Vidua* have the evolutionary potential to modulate spot size in accordance with selection pressures.

It is more questionable whether the absence of bill tip pattern mimicry in the Pin-tailed Whydah – Common Waxbill parasite-host pair could be due to genetic constraint. All the photos of *Vidua* young from different species show a dark wedge at the bill tip (e.g. see photos of Vidua in Figure 3 of Payne 2005b). None show the fine, double-line pattern exhibited by Common Waxbills. Therefore, its absence in Pin-tailed Whydah nestlings may reflect some constraint.

At a broader scale, however, the lack of *Vidua* species colonising certain hosts may be due to genetic constraints preventing them from evolving the necessary mimetic mouth markings. For example, mannikins (*Spermestes* spp.), the Locust Finch (*Paludipasser locustella*) and several other African estrildid species (e.g. *Euodice* silverbill species) have one or two horizontal bars on the upper palate rather than spots (Figure 4.6, and Chapter 6). However, none of these estrildids are regularly parasitised by a *Vidua* species. This is despite some species, such as Bronze Mannikin (*Spermestes cucullatus*), being common in habitats where *Vidua* occur, and Pin-tailed Whydah eggs occasionally being found in their nests (Tarboton 2011, personal observations). The reason for this lack of parasitism by *Vidua* could be that *Vidua* finches have not been able to evolve the palate bar markings necessary to successfully exploit them. However, palate bars have evolved independently five times in estrildid finches (based on ancestral state reconstructions in Chapter 6), suggesting that the trait is reasonably labile. Given that the Viduidae and Estrildidae are sister families it may be that *Vidua* also possess the necessary genetic architecture to evolve palate bars if the selection pressures were to arise. If this were true, it would count against the "constraint" hypothesis.

3) Parasite signals are improved versions of host signals which are better at manipulating host parents ("super-stimulus")

It is possible that the larger overall spot size in Pin-tailed Whydahs represents a "super-stimulus" that manipulates host parents to feed Pin-tailed Whydahs more than

they would otherwise feed their own young. That it is only the distal three spots that are larger also supports this hypothesis, as these are the three spots will receive most light from the surroundings and that are most exposed and visible to parents. Therefore, it is likely that selection from host parents will be stronger on these three spots than on the two spots further down in the mouth.

Alternatively, the front three spots might be enlarged to create on optical illusion. If the front three spots are bigger than the back two, this could create an illusion of increased distance between the front and back spots. It is possible that this could draw the parent's eyes into the centre of the chicks mouth and so make the parent more likely to feed that chick. Similar optical illusions have been shown to be used by male Great Bowerbirds (*Ptilonorhynchus nuchalis*) to draw in female bowerbirds (Kelley and Endler 2012). Here males position objects on their display court so that larger objects are located further away from the bower. This size gradient creates an illusion of a larger display avenue leading to the bower, and results in increased mating success for the males.

At this stage, suggestions that mouth marking discrepancies in parasitic nestlings compared to hosts serve as a super-stimulus are purely speculative. To test whether they do function in this way, experiments would have to be done in which the mouth markings of host nestlings are manipulated in the direction of parasites (larger distal spots, more black on bill tip) and the effects on parental feeding rate, chick growth and survival monitored.

4) Evolutionary lag

The "evolutionary lag" hypothesis, that parasites have not yet had the time to respond to selection pressures from host is unlikely to apply in the context of Pin-tailed Whydah and Common Waxbills. Pin-tailed Whydah are thought to parasitise Common Waxbill across a very large range in Africa, ranging from Cape Town in the south north to Ethiopia in the east and Sierra Leone in the west. Therefore, Pin-tailed Whydahs have likely been parasitizing Common Waxbills for a long time and there has likely been adequate time to evolve more precise mimicry were it being selected for.

Evolutionary lag is, however, likely to account for imperfect mimicry of hosts by *Vidua* in a few situations which are likely the result of recent colonisations of new hosts. In southern Zambia, there is a population of Village Indigobird that is parasitizing Brown Firefinch (*Lagonosticta nitidula*) but retains the mouth markings of its ancestral host, Red-billed Firefinch (Payne et al. 2002). Similarly, in Cameroon, Pin-tailed Whydahs have been recorded parasitizing Black-crowned Waxbills, but retain mouth markings matching those of Common Waxbill (Lansverk et al. 2015).

It should be noted that the evolutionary lag hypothesis is just an extreme case of the genetic/developmental constraints hypothesis. Essentially, the hypothesis implies that the necessary genetic variation does not yet exist in the population to respond to selection appropriately. If constraints are very strong, this means that the likelihood of the appropriate mutations occurring is very low and it is likely to take a long (effectively infinite) amount of time for the necessary variation to arise. Additionally, the evolutionary lag hypothesis suffers from being essentially unfalsifiable in that it is always possible to invoke the absence of an adaptation as being due to there not having been enough time (Kilner and Langmore 2011).

Further work on visual mimicry

In this chapter I have presented quantitative evidence on pattern mimicry between *Vidua* and host mouth markings (Figures 4.7, 4.8 and 4.9). I have also presented quantitative evidence for imperfect mimicry in between *Vidua* and host mouths (Figure 4.10). Future work to quantify this similarity could use a multinomial logistic regression approach as I employed to quantify the begging call mimicry. This approach could be extended to analyse colour in addition to pattern mimicry. Here, cone-capture values for different mouth marking features of each locally occurring estrildid finch species would be extracted. These would be entered into a multinomial logistic regression model to generate a classification function. Subsequently, parasite mouth values would be fed into the classification function and the accuracy with which the model assigned the parasite to its correct host would be assessed.

Vocal mimicry

The multinomial logistic regression, discriminant function analysis and the human assessment trials all provide evidence that the three species of *Vidua* nestlings

investigated all mimic the begging calls of their respective hosts (see Figures 4.13, 4.14 and 4.15 for example sonograms).

Comparisons of the "we-chee" calls (call types 4a and 4b) of Pin-tailed Whydah and Common Waxbill revealed that the second element (4b) is significantly longer in Pin-tailed Whydahs. This discrepancy is clearly audible when listening to the two species beg and when viewing the sonograms (Figure 4.13). It is possible that these longer call notes by parasite chicks represent a "super-stimulus", eliciting greater parental care from host parents than a host chick would be able to. In order, to test this hypothesis, playback experiments would have to be carried out in which the calls of parasite chicks are broadcast at host nests and the feeding rates of host parents compared to when host chick calls are played.

Of the five individual Pin-tailed Whydah nestlings tested for mimicry, four were raised without Common Waxbill nestmates. This suggests that the vocal mimicry shown is not learned from interactions with host nest mates. Additionally, three of the five Pin-tailed Whydahs included in the analysis had been raised in Blue Waxbill nests as part of the transfer experiments carried out in Chapter 5. Despite this, two of these three still developed begging calls that were assigned to Common Waxbill by the multinomial logistic regression and discriminant function analysis models. The one individual whose calls were not assigned to Common Waxbill were instead miss-assigned to Bronze Mannikin and not to Blue Waxbill. This suggests that begging call mimicry is not learned from interactions with host parents as is the case in Horsfield's Bronze Cuckoo (*Chalcites basalis*) (Langmore et al. 2008). Whilst the sample sizes are still small, both these findings suggest that begging call mimicry by Pin-tailed Whydahs is innate and not requiring guidance in development from host nest mates or parents (see Chapter 5). In this chapter, I focussed only on calls of nestlings in mid to late development, and not on the calls given by chick in the first half of the nestling period. Further work could extend the analysis to earlier stages to see at what stage mimicry begins and whether it is modulated by the presence or absence of nestmates.

Postural mimicry

The descriptive accounts of movements made during begging suggest that mimicry between *Vidua* and hosts might also occur in these traits. Common Waxbill was the only of the 8 estrildid finch species observed at our field site which did not move its head whilst begging. Similarly, its parasite, Pin-tailed Whydah, was the only one of the three *Vidua* species measured not to move its head when begging. This absence of head movement is contrary to the Pin-tailed Whydah species description in *Roberts' Birds of Southern Africa* (Hockey et al. 2005), which states that the "young whydah solicits food with distinctive side-to-side swaying motion, mimicking young waxbill's soliciting action". Whilst this is true for Broad-tailed Paradise Whydah, it is not true for Pin-tailed Whydah. This statement is also false because Common Waxbills do not sway their heads side-to-side when begging. Hockey et al. (2005) cite Nicolai (1964) when making this claim. It is possible that Nicolai was describing the begging display of other whydah species and this was generalised to another whydah species

Newly-hatched Pin-tailed Whydahs do differ from Common Waxbills in an important aspect of their begging movements. Specifically, Pin-tailed Whydahs wave one of their wings slowly up and down when begging. Only wing arm on the side of the chick's body that the open mouth is facing towards is waved. This behaviour is not present in older chicks. The wings of Pin-tailed Whydahs have several distinctive white, hair-like feathers which Common Waxbill chicks lack. It is possible that these white feathers serve to further stimulate host parents to feed them in the way that the yellow wing patches on Horsfield's Hawk-cuckoos (*Hierococcyx fugax*) (Tanaka and Ueda 2005). The white feathers against the dark skin of the Pin-tailed Whydah nestling could appear similar to the contrast between the white outer and black inner gape flanges that line the mouth. Further work could test this by trimming off the wing feathers of nestling Pin-tailed Whydahs and seeing what effect this has on host feeding rate, nestling growth and survival.

The sequence of evolving multimodal mimicry

When a *Vidua* finch or any specialist parasite colonises a new host it will need to mimic several host traits if it is to thrive in its new environment. This chapter shows that at least some *Vidua* species have evolved mimicry of multiple components of host begging displays. This raises the question of, when colonising a new host, which

mimetic traits evolve first. In the context of *Vidua*, do parasites converge on host begging calls, head movements or mouth markings first? Which of these traits are most important in soliciting care from host parents? It is possible that traits which are capable of more plasticity in development have the potential to evolve faster. If this was the case, we would predict that *Vidua* colonising new hosts would converge on plastic, behavioural traits such as begging calls and head movements and only converge on mouth markings in subsequent generations. To investigate this, it would be interesting to measure the traits of parasites that have recently colonised new hosts. Examples include the population of Village Indigobird that has recently started parasitising Brown Firefinch in southern Zambia. The mouth markings of this indigobird population are known not to match those of the new host (Payne et al. 2002). The begging calls and head movements in this population, by contrast, have not been measured. Similarly, the population of Pin-tailed Whydah parasitising Black-crowned Waxbill in Cameroon would also be worth investigating (Lansverk et al. 2015).

Chapter 5: *Limits to host colonisation and speciation in* Vidua *finches*

Chapter 5:
Limits to host colonisation and speciation in *Vidua* finches

5. 1. INTRODUCTION

A key trade-off faced by parasites is that adaptations making them effective at exploiting one host can simultaneously make them less suitable at exploiting another (Price 1980). As a parasite becomes better adapted to a certain host, the range of other hosts it can potentially colonise may diminish (Agudelo-Romero et al. 2008; Duffy et al. 2006). Consequently, parasites can become dependent on a single host and vulnerable if that species goes extinct or evolves counter-defences. When colonisation of new hosts is closely linked to parasite speciation, understanding the factors that determine host suitability and limit host colonisation becomes key to explaining the diversification of a parasite clade. If parasite lineages speciate each time they colonise a new host, the species richness of the parasite clade will be directly related to the number of hosts it can successfully colonise (Poulin and Morand 2000).

Close associations between speciation and host-switching have been found in several groups, including phytophagous butterflies (Hardy and Otto 2014), *Rhagoletis* flies (Bush 1969; McPheron et al. 1988) and fish ectoparasites of the genus *Gyrodactylus* (Ziętara and Lumme 2002). Among vertebrates, the best example of speciation via host-switching comes from the indigobirds and whydahs (genus *Vidua*) of Africa. The genus *Vidua* is a radiation of 19 brood-parasitic finch species. They are almost all host specialists, each parasite species laying its egg in the nest of a single host species of the grassfinch family (Estrildidae). Estrildid finches vary dramatically in their nestling phenotypes. Each species' young have a characteristic combination of mouth markings, skin colour, begging calls, head movements and patterns of natal down (see Chapter 4 and 6). As shown in Chapter 4, the nestlings of *Vidua* species have evolved mouth markings, begging calls and head movements which mimic those of their host.

Host colonisation and speciation in Vidua finches: the role of imprinting

Speciation is linked to host colonisation in *Vidua* finches because of their remarkable capacity to imprint on hosts (Sorenson et al. 2003). Male *Vidua* finches incorporate elements of their host species' vocalisations into their own displays as adults (Payne et al. 1998). Female *Vidua* finches are attracted to males who sing like the host that she was raised by, and also show a preference to lay eggs in a nest of that same host species (Payne et al. 2000). Therefore, male display, female mating preference and female host preference are all strongly influenced by the host environment the bird developed in. The result is that male and female *Vidua* raised by the same host tend to breed with one another and parasite-host associations are maintained across generations (Payne et al. 2000).

This imprinting mechanism means that, if a female lays her egg in the nest of a previously unparasitised host species and her offspring survive in the new host environment, they have the potential to initiate a new lineage of *Vidua* (Sorenson et al. 2003). Male offspring will sing like the new host, while female offspring will be attracted to males who sing like the new host and will subsequently prefer to parasitise the new host. Imprinting therefore promotes positive assortative mating by host rearing environment, and can thus generate reproductive isolation between *Vidua* associated with different hosts over the course of a single generation. Genetic studies have shown that *Vidua* species are of much more recent origin than their hosts, supporting a model of speciation via host colonisation rather than by co-speciation with hosts (Klein and Payne 1998; Sefc et al. 2005; Sorenson et al. 2004; Sorenson et al. 2003). The existence of a population of Village Indigobirds (*V. chalybeata*) parasitizing and vocally imitating Brown Firefinches (*Lagonosticta nitidula*) in southern Zambia, despite possessing the nestling mouth markings of their ancestral host the Red-billed Firefinch (*L. senegala*), suggests that this is a recent host colonisation and provides further evidence for the model (Payne et al. 2002). Additionally, observations of hybrid *Vidua* suggest that females occasionally lay in the nests of other estrildid hosts, some of which are already being used by other *Vidua,* potentially setting the stage for introgression as well as speciation (Payne 1980).

Patterns of host colonisation in *Vidua* are not, however, random. *Vidua* primarily colonise only hosts in the same clade (and usually the same genus) as their ancestral host, in a pattern termed "clade-limited colonisation" (Sorenson et al. 2004). If we can understand what limits successful colonisations to only certain estrildid hosts and not to others, we can begin to explain why the *Vidua* radiation has diversified to just 19 species, rather than many more or fewer.

Conditions for successful host colonisation by Vidua

There are several conditions that must be met to allow successful colonisation of a new host by a species of *Vidua*: (i) the geographical ranges of host and parasite must overlap; (ii) the female *Vidua* must at least occasionally lay eggs in the potential host's nest; (iii) the *Vidua* offspring must hatch in and survive to fledge from the new host environment; and (iv) the offspring must survive to adulthood and find a mate who has been raised by the same host. All four conditions must be met for successful host colonisation to occur and a new lineage of *Vidua* to become established.

Patterns of range overlap between host and parasites alone are unable to explain why only some estrildid finch species are parasitized by *Vidua*. This is because, at a given locality, not all estrildid finch species are parasitized. For example, at our field site in southern Zambia, there are six species of estrildid finch that are regularly parasitized by *Vidua* and six species which are rarely or never parasitized. The six parasitised species are Jameson's Firefinch (*Lagonosticta rhodopareia*), Red-billed Firefinch (*L. senegala*), Melba Finch (*Pytilia melba*), Orange-winged Pytilia (*Pytilia afra*), Common Waxbill (*Estrilda astrild*) and Red-throated Twinspot (*Hypargos niveoguttatus*). The six unparasitised estrildid species at the site are Blue Waxbill (*Uraeginthus angolensis*), Bronze Mannikin (*Spermestes cucullatus*), African Quailfinch (*Ortygospiza atricollis*), Locust Finch (*Paludipasser locustella*), Orange-breasted Waxbill (*Amandava subflava*) and Cut-throat Finch (*Amadina fasciata*). Interestingly, both African Quailfinch and Orange-breasted Waxbill are parasitized by specialised indigobird species in west Africa (Quailfinch Indigobird, *V. nigeriae*, and Jambandu Indigobird, *V. raricola*, respectively) but not in southern Africa (Fry and Keith 2004), suggesting that neither species is intrinsically unsuitable for colonisation. Importantly, all the unparasitised estrildid finches, apart

from the Locust Finch, breed commonly at the study site and in similar habitats to the parasitized species.

Vidua females do, however, occasionally lay eggs in the nests of estrildid species that are not primary hosts. During my fieldwork in Choma, Zambia, from 2013–2017, a single Blue Waxbill nest and two Bronze Mannikin nests were found to naturally contain a Pin-tailed Whydah egg, suggesting that these species are occasionally parasitized. This observation concurs with the presence of Pin-tailed Whydah eggs in Bronze Mannikin and Zebra Waxbill nests in egg collections from Zimbabwe (Duncan Parkes *pers. comm.*) and references to Pin-tailed Whydahs occasionally laying in the nests of several other estrildid species (Meredith and Mullers 2015; Tarboton 2011).

Therefore, despite overlapping in range with *Vidua* species and occasionally having a *Vidua* egg in their nest, some estrildid species still do not become regular hosts. Consequently, poor offspring survival in novel host environments and/or inability to find mates once adult must be important. Tracking parasite young to adulthood and examining mating patterns is logistically challenging. Additionally, the first hurdle the chick must overcome is to survive in and fledge from a foreign nest. Therefore, it is this part of the colonisation process that the chapter focuses on.

Hypotheses

Parasite chicks may fail to successfully fledge from a host nest for several reasons. First, the host could have evolved egg rejection abilities and remove or not incubate foreign eggs. All *Vidua* and estrildid finch species lay white eggs. This is likely not due to convergent evolution but instead due to shared ancestry. The Viduidae and Estrildidae are sister families (approximately 15-16 million years divergent (Gomes et al. 2016)) and their common ancestor is thought to have had white eggs (Sorenson et al. 2003). Therefore, it is unlikely that host parents can use visual cues to distinguish host from parasite eggs unless there are cryptic differences in the ultraviolet spectrum as was found in Pallid Cuckoos and their hosts (Starling et al. 2006).

Second, at the chick stage, host parents may detect odd-looking young, and either remove them from the nest or provide them with less food than they would their

own chick. Both egg rejection and chick rejection have evolved in the hosts of several other brood parasites (Langmore et al. 2003; Langmore and Spottiswoode 2012). Additionally, hosts may feed their nestlings a specific diet which differs from that which the parasite is used to, causing the latter to have poor growth and survival (Davies and Brooke 1989).

These barriers to host colonisation can be summarised by the following hypotheses:

Hypothesis 1: Host parents reject foreign eggs

Hypothesis 2: Host parents reject and remove foreign chicks

Hypothesis 3: Foreign chicks are not actively rejected by host parents, but survive less well than the host's own chicks do.

If hypothesis 3 is true, there are several mechanisms which could drive it. These include:

Hypothesis 3.1: Host parents feed foreign chicks less food than they feed their own chicks.

Hypothesis 3.2: Host parents feed foreign chicks a different type of food than the foreign chick is normally fed in its natural nest.

Parasite adaptations to novel host environments: begging call plasticity

If hypothesis 3.1 is true, it could be because the new host parents feed their young less food overall than the natural host does. Alternatively, it could be because foreign chicks are specifically being fed less food than hosts chicks are. This could result from a mismatch in the begging displays of foreign and host chicks. Begging displays in *Vidua* and estrildid finches have three components: mouth markings, begging calls and head movements (see Chapter 4). In Chapter 4, I provided evidence that at least three *Vidua* species mimic their natural hosts in all these aspects of host begging display. Therefore, when colonising a new host with different begging displays, there

will be a mismatch between the begging displays of the host and that of the *Vidua* nestling.

While nestling mouth pattern is likely to be a fixed trait, it is possible that begging calls and head movements could develop plastically depending on host environment. Other avian brood parasites, such as the Horsfield's Bronze-cuckoo (*Chalcites basalis*), have been shown to be able to adjust begging calls plastically depending on their host environment (Langmore et al. 2008). However, there are reasons to expect that *Vidua* finches will not show major differences in begging call structure depending on the host environment they are raised in. This is predicted by the conceptual framework on begging call development put forward in Chapter 2. *Vidua* finches are host specialists and colonisation of new hosts is rare. This means that a finch's ancestry, as coded in its genetics, strongly predicts the host environment a *Vidua* nestling will experience. Given that host species is accurately predicted by genetic cues, additional environmental cues for call development are redundant and plasticity should not evolve. This would be the case especially if there is a cost to maintaining plastic begging call development. These costs could be physiological, but could also be behavioural if plasticity can lead to *Vidua* accidentally developing the wrong displays due to random environmental perturbations in the developmental process. Another highly specialised brood parasite, the Screaming Cowbirds (*Molothrus rufoaxillaris*), which mimics the begging calls of its host, showed no plasticity in begging calls when transferred into a non-host nest (De Mársico et al. 2012). Thus, a fourth barrier to host colonisation can be summarised by the following hypothesis:

Hypothesis 4: Parasitic nestlings don't develop different begging calls in different host environments.

To re-create the colonisation of a novel host species, I experimentally transferred eggs of Pin-tailed Whydahs into the nests of an estrildid species, the Blue Waxbill. Blue Waxbills are extremely common at our field site and yet are almost never used as a host by a *Vidua* species. The Pin-tailed Whydah's range overlaps widely with that of the Blue Waxbill, and there is evidence that female Pin-tailed

Whydahs do occasionally lay in the nests of Blue Waxbills (Hockey et al. 2005; Tarboton 2011, personal observations).

Pin-tailed Whydah nestlings raised in their natural host nest differ markedly from Blue Waxbill nestlings in mouth markings (Figure 5.1), head movements, and begging calls (see Chapter 4). This suggests that survival of foreign young in Blue Waxbill nests may be a factor limiting colonisation of this potential host species. If this is true, we would predict that Pin-tailed Whydahs raised in Blue Waxbill nests would survive much worse than Blue Waxbill nestlings raised in Blue Waxbill nests. Additionally, we would expect that Common Waxbill nestlings, which are the natural hosts of Pin-tailed Whydahs and possess similar begging displays, would also survive poorly in Blue Waxbill nests. If Pin-tailed Whydahs possess additional adaptations to survive in foreign nest environments, such as plasticity in key traits or tolerance of a greater diversity of nestling diets, we would expect Pin-tailed Whydahs to survive better than Common Waxbills do in Blue Waxbill nests.

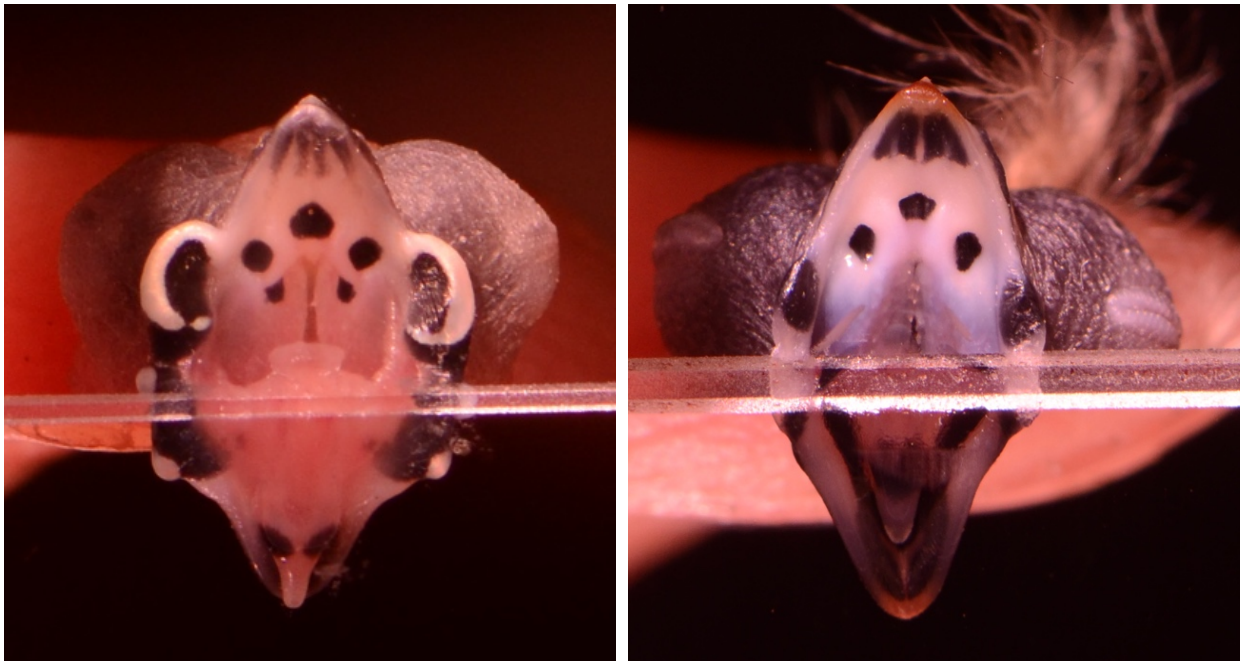


Figure 5.1. Mouth marking photos of newly-hatched nestling Pin-tailed Whydah (left) and Blue Waxbill (right).

Figure 5.2. Pin-tailed Whydah nestling that had been transferred to a Blue Waxbill nest with a large crop (score = 3).

Figure 5.3. Sampling the crop contents of a Pin-tailed Whydah nestling using the tube insertion method of Zann & Straw (1983).

The transfer experiment had three treatments: (i) Pin-tailed Whydah eggs transferred to Blue Waxbill nests, (ii) Blue Waxbill eggs transferred to Blue Waxbill nests, and (iii) Common Waxbill eggs transferred to Blue Waxbill nests. The second treatment acted as a positive control: if Blue Waxbills survive poorly in Blue Waxbill nests other than their own, it would suggest that Blue Waxbill parents could be discriminating against any foreign eggs/chicks more generally and not against chicks with mismatching begging calls, mouth markings and head movements specifically. Alternatively, it could also suggest the artificial incubation and/or transfer process reduced chick viability in some way.

The third treatment allowed me to test whether Pin-tailed Whydahs possessed any additional parasite-specific adaptations that allow them to survive in novel host environments. Common Waxbills are the natural hosts of Pin-tailed Whydahs, and possess very similar begging displays including mouth markings, calls and behaviour (see Chapter 4). If Pin-tailed Whydahs survive better than Common Waxbills do in Blue Waxbill nests, it

suggests that they possess some additional adaptations which allow them to survive in a novel host environment despite having mismatching begging displays.

5.2 METHODS

Transfer experiments

During January–May 2014, 2015, 2016 and 2017, I carried out transfer experiments within an area of about 40 km^2 on and around Musumanene and Semahwa Farms (centred on 16°47′S, 26°54′E) in the Choma District of southern Zambia.

I moved Pin-tailed Whydah, Common Waxbill and Blue Waxbill eggs or chicks into Blue Waxbill nests. In addition, a small number of Jameson's Firefinch eggs (n = 2) were added to Blue Waxbill nests as part of a preliminary study to see whether the results from Common Waxbill and Pin-tailed Whydah chicks generalised to species with different begging displays. However, it was subsequently decided to focus efforts on increasing sample sizes for the core treatments rather than to increase the overall number of treatments. Therefore, the sample size of Jameson's Firefinch transfers remained small and the data were only incorporated in testing the egg rejection hypothesis.

To minimise predation risk, eggs were taken from their natural nest and stored in a Brinsea Octagon 20 Advance EX Incubator at 36.7°C and 60% humidity. Eggs were retained in the incubator until a day or two before they were due to hatch, before being transferred to a recipient Blue Waxbill nest. This was done to reduce the probability that the egg would be transferred to a nest that would subsequently get predated.

The incubation stage was estimated by 'candling', in which a torch is shone through the back of the egg to better see the contents. Eggs were scored as being: "0" (fresh, no sign of embryo), "1" (small embryo visible), "2" (embryo larger and vascularisation spreading around most of the one side of the egg), "3" (vascularisation has spread to over half of the egg), "4" (embryo large such that light only comes through one quarter of the egg when candled), "5" (embryo very large and egg about to hatch within a day or two; no light comes through the egg when candled). Eggs were only transferred to recipient Blue Waxbill nests when eggs were at incubation stage 4 or 5.

For 17 of the 98 total transfers done, the foreign offspring was transferred as a chick freshly hatched from the incubator rather than as an egg. This was done to allow the mouth marking of the newly-hatched chick to be photographed (see Chapter 4) before the chick transferred, increasing sample sizes for both mouth marking and survival analyses. Whether the propagule was transferred as an egg or a newly-hatched chick was controlled statistically in the survival analysis models (see below).

Recipient Blue Waxbill nests were chosen from amongst the Blue Waxbill nests which were active at the time and I attempted to synchronise the developmental stage of the transferred egg with that of the eggs in the recipient nest. However, when this was not possible, the egg was transferred to a nest at an earlier developmental stage to the transferred egg. The transfer egg was simply added to the recipient nest and no host egg removed. This mirrors the behaviour of Pin-tailed Whydah (and *Vidua* more generally) females in the wild, which usually do not remove a host egg when laying one of their own (Tarboton 2011, personal observations).

Experimental nests were visited every two days (occasionally every three days), and the number of eggs and chicks in the nest were recorded. For eggs, the dimensions were measured on the first visit and the incubation stage recorded on every visit. For chicks, the mass and tarsus length were measured and the amount of food in the crop scored (see below for details). Mass was measured on digital scales in grams to an accuracy of 0.1 or 0.01g depending on the model of weighing scale used. Tarsus length was measured using dialMax Vernier Dial Callipers to the nearest 0.1 mm.

Comparing survival of different species transferred to Blue Waxbill nests

To compare survival of transferred chicks of different species in Blue Waxbill nests, analyses were carried out in the R statistical environment (R Development Core Team 2017) using the packages Survival (Therneau 2015) and KMsurv (Moeschberger and Yan 2012).

Survival analyses model the time it takes for an event to occur. In the context of these transfer experiments, the event of interest is the death of the transferred chick. This is not death due predation of the nest, but rather the specific death of the transferred chick even though other members of the brood remain alive. The period of chick survival was judged to

begin on the day the chick hatched in the new host nest, and to end at the midpoint between the last day the chick was known to be alive and the first day the chick was known to be absent. If the nest was still active at the point the transferred chick was absent, a "death" event was deemed to have occurred. If the nest was abandoned at the point the transferred chick is absent (e.g. due to predation of the nest), the data are "right-censored". This means that the chick was deemed to have survived at least this long, but could have survived longer were it not for the predation event. This allows survival data in which the outcome is uncertain still to be included in the analysis.

A Cox proportional hazards model was fitted to the survival data. This is a semi-parametric model because, whilst it makes no assumptions about the shape of the hazard function (the risk of an event occurring at a time point conditional on that event not having occurred up until that point), it does assume that the co-variates influence survival in a linear manner. There was *a priori* no reason to expect that the co-variates influenced survival non-linearly so this was considered a valid simplifying assumption.

The co-variates included in the initial model were: (i) the species of chick that had been transferred (i.e. treatment type), (ii) the presence or absence of host nestmates, and (iii) whether the foreign chick had been transferred as an egg or as a chick. The presence of nestmates could influence the survival of transferred chicks because they would have to compete with more chicks to be fed by host parents. The number of nestmates in the nest over the course of a given transfer experiment ranged from a minimum of 0 to a maximum of 5. The mean number of nestmates was 1.4 and the median was 0. In most transfers, (56 out of 105 total) the transferred chick did not have any nestmates in the nest. Given this, it was decided to model the presence of nestmates as a binary, presence/absence, variable rather than as a continuous variable. Whether the chick was initially transferred as an egg or a chick was included as a co-variate because it is possible that host parents will react differently to the sudden appearance of a new chick in the nest rather than the sudden appearance of a new egg. In case this influenced parental discrimination behaviour, it was initially included in the model.

Once the initial model was run, the AIC values of the full model were compared with the AIC values of a null model (a model comprising only the intercept) (Burnham and Anderson 2001). Comparing the two gives an overall significance level for all the predictors

together, while avoiding the problem of multiple testing. Subsequently, the AIC values of models in which each of the co-variates is dropped (reduced models) was compared with the AIC of the full model to examine which factors have a significant impact on the response variable.

Comparing the amount of food host parents fed transferred chicks of different species

To measure how much transferred chicks were being fed, the crop size of the transferred chick each time we visited the nest was recorded. Crops of nestling estrildid finches are transparent and allow easy external visual inspection. Crops were scored as 0 (empty), 1 (trace amounts, < c. 20 seeds, and with no bulge in crop), 2 (> c. 20 seeds and with slight bulge) or 3 (> c. 50 seeds and with large bulge) (Figure 5.2).

To assess whether crop sizes of chicks differed depending on the species of chick transferred, two approaches were used. First, the median crop size of the transferred chick over the first 7 days of survival in the host nest was measured and used as the response variable. Only the crop scores over days 0 to 7 were included because this is the period over which c.80% of the Common Waxbill and Pin-tailed Whydah chicks transferred died (Figure 5.4), and so is the relevant window in which to compare parental feeding behaviour among species. Given the non-normal nature of the crop score response variable, a Kruskal-Wallis test was carried out to test whether median crop sized differed between the three species. As nests were visited at random times in the day with respect to the identity of the chick (i.e. we were not more likely to visit a transferred Pin-tailed Whydah in the morning than a transferred Blue Waxbill), median crop size should give us a sense of how well fed the chick was over the course of the experiment. A Dunn post-hoc test was carried out to compare median crop size of each transferred species to one another using the dunnTest function from the R package FSA (Ogle 2017), with Bonferroni correction for multiple testing.

In the second approach, ordinal mixed-effect models were employed with the R package Ordinal (Christensen 2015) with crop score as an ordinal response variable. In the full model, the fixed effects are chick species and the number of nestmates. Transferred chick individual was a random effect nested within the nest of origin of that transferred chick. I carried out stepwise elimination of non-significant co-variates until only significant co-variates remained. The mixed-effects approach allowed more of the data to be used than the approach in which median crop score is taken. The model was initially run to include crop

scores over the first 7 days of development (as was done for the Kruskal-Wallis test above). Subsequently the model was re-run using crop scores over the first 4, 5, 6 and 8 days of development to see how robust the findings from the first 7 days of development were.

To test whether the amount of food the transferred chick was fed explained variation in chick survival, another Cox proportional hazards model was fitted to the survival data with median crop size over the first 7 days of development used as an explanatory variable.

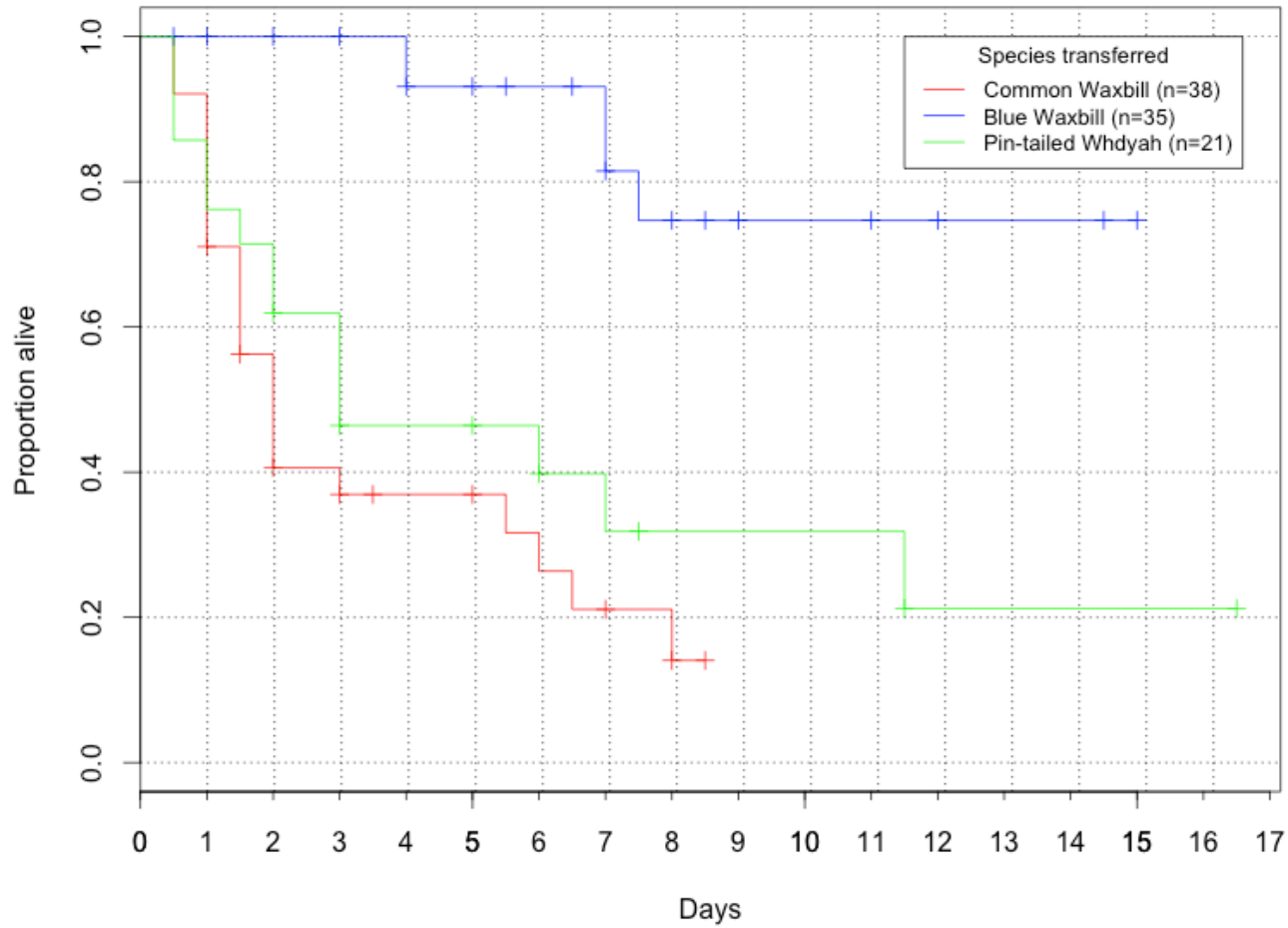


Figure 5.4. Survival curves for Common Waxbills (natural host), Pin-tailed Whydahs (parasite) and Blue Waxbills (novel host) transferred to Blue Waxbill nests. Blue Waxbills were found to survive significantly longer in Blue Waxbill nests than either Common Waxbills (survival analysis, $z= 4.709$, $p < 10^{-5}$) 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, $z= 1.247$, $p> 0.2$).

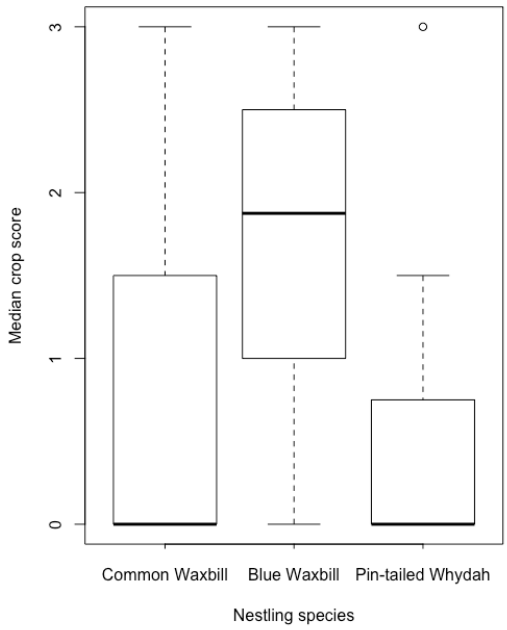


Figure 5.5. Median crop size from day 0 to day 7 for chicks that had been transferred to Blue Waxbill nests. Median crop size significantly differed between species (Kruskal-Wallis chi-squared = 14.2, $p < 0.001$)

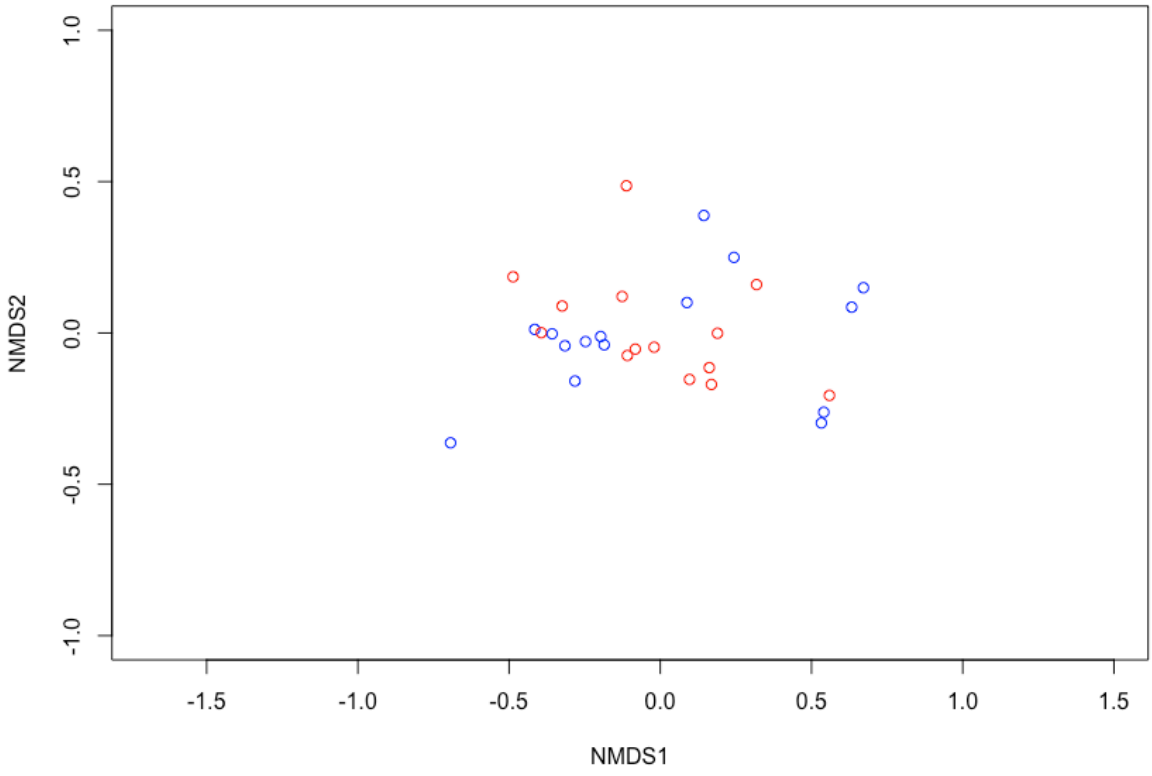


Figure 5.6. Niche partitioning in nestling diet at the subfamily level in Blue Waxbill (blue circles) and Common Waxbill (red circles) nests. NMDS of Bray-Curtis dissimilarities (adonis pseudo $F = 5.347$, $p < 0.05$)

Measuring nestling diet composition

Obtaining crop samples in field

Nestling crops were sampled using the tube insertion method (Zann and Straw 1983). A tube previously used to carry bird rings (measuring about 2.7 mm external and 1.8 mm internal diameter) was used. The tube was inserted down the throat of the nestling until the tube became visible through the skin of the throat. Seeds were then pushed into the tube from the outside using a finger (Figure 5.3). The tube was withdrawn and the contents blown out into a vial containing 70% ethanol. The process was repeated until about 20–30 seeds had been extracted. This method does not harm the chicks, and allows crop samples to be obtained on multiple days in development. The age of the chick being sampled was standardised as much as possible to be around the time when the primaries first erupt from pin (approximately day 5 to 7). This was around the earliest stage in development that the crops could be sampled using the tube insertion method. Before that time the chicks were too small, and the tube could not be put down their throats without risk of damaging the chick. It also represents the period at which most Common Waxbills and Pin-tailed Whydahs transferred to Blue Waxbill nests died, so could give us an insight into what dietary differences were operating at this stage.

DNA barcoding of nestling crop contents

Nestling estrildid finch crops sampled were found to obtain almost exclusively plant seeds, occasionally mixed with some ants (Formicidae) and termites (Isoptera). DNA barcoding of samples was carried out by the company Jonah Ventures (Boulder, Colorado; jonahventures.com). Initially, I asked them to sequence both the insect and plant components of the diet. However, only the plant component successfully amplified. It is possible that the 70% ethanol the seeds were stored in did not preserve the insect DNA well enough. For this reason, as well as because of cost limitations, and because the clear majority of the crop contents are made up of plant seeds, I focussed on characterising the plant component of the nestling diets.

Jonah Ventures carried out the sequencing and bioinformatics analysis under the following protocol:

Amplification and sequencing of DNA from diet samples

The chloroplast trnL intron was PCR amplified from each DNA sample using the c and h trnL primers. Amplification primers also contained a 5' adaptor sequence, allowing for subsequent indexing and Illumina sequencing. Each 40 µL PCR reaction was mixed per the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 0.4 µM of each primer and 3.2 µl of gDNA. The following conditions were used for amplification: initial denaturation at 94 °C for 1 minute, followed by 36 cycles of 1 minute at 94 °C, 30 seconds at 55 °C, and 30 seconds at 72 °C, and a final elongation at 72 °C for 1 minute.

Amplicons were then cleaned using the UltraClean-htp 96-well PCR Clean-up kit and stored at 4 °C. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 uM of each primer and 4 µl of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds. After trnl-specific and indexing PCR reaction, 5 µl of PCR products of each sample were visualized on a 2% agarose gel. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). Sequencing was carried out on an Illumina MiSeq (San Diego, CA) in the CU Boulder BioFrontiers Sequencing Center using the v2 300-cycle kit (cat# MS-102-2002).

Assigning taxonomy to the sequenced DNA

After sequencing, the trnL amplicons were processed via the UPARSE pipeline (Edgar 2013) and assigned taxonomy via the UTAX protocol (http://www.drive5.com/usearch/manual/utax_user_train.html) available in usearch (v8.1.1861) (Edgar 2013). Further filtering of any primers and adapter regions that remained were removed using cutadapt (Martin 2011). Sequences were quality trimmed to have a maximum expected number of errors per read of less than 0.5.

To assign taxonomy to each operational taxonomic unit (OTU), a UTAX trnL reference database was constructed by downloading any annotated GenBank (Benson et al. 2005) records that contain the trnL gene. The amplicon region bounded by the trnL c & h

primers (Taberlet et al. 2007) was extracted from the GenBank records using the UTAX protocol. Closed-reference OTUs were generated by searching against the trnL reference database at 99% sequence similarity. Additional OTUs were generated when sequencing plant voucher specimens that were collected privately by Jonah Ventures, to supplement the set of species present in GenBank.

Validating the taxonomies assigned to OTUs

To validate whether the species, genus, subfamily and family level assignments of taxonomy to the OTUs from the DNA barcoding experiment were realistic, I conducted opportunistic grass surveys to characterise the locally abundant grass species. Additionally, I sent the list of assigned taxa to an expert botanist based in Zambia, Mike Bingham. He validated which taxa occurred commonly in the Choma area and, of those, which produced seeds that would be potentially palatable for an estrildid finch.

Analysing the crop contents data

Samples obtained from multiple chicks in the same nest on the same day were pooled together to give a single sample. If a nest had been sampled on multiple days, the sample from a single day was chosen at random.

Once each OTU was given a taxonomic identification, it became clear that the resolution of the data was only good enough to analyse at the subfamily level and not the genus level (see the Results section for more details).

Each OTU was assigned to one of the four subfamilies identified (again see Results for details of the sub-families). For each nestling sampled, reads from OTUs mapping to the same sub-family were summed together to give a measure of the total number of reads from each subfamily. This was then divided by the total number of reads for that sample to obtain the proportion of reads mapping to each of the four subfamilies for that sample.

To see whether different parent species fed chicks different proportions of seeds from each of the four families, non-metric dimensional scaling (NMDS) was performed using the R package vegan (Oksanen et al. 2017). NMDS was preferred over PCA because NMDS uses ranks rather than absolute values, making it more suitable for analysing proportions. Comparisons of diet between species were made using the function adonis, also from the R

package vegan (Oksanen et al. 2017), which carries out a multinomial analysis of variance (MANOVA) using estrildid parent species as a categorical predictor variable.

Begging call plasticity

Recording nestling begging calls

To assess whether transferred Pin-tailed Whydah nestlings developed begging calls differently depending on whether they were raised in their natural host's nest (Common Waxbill) or in the non-natural host nest (Blue Waxbill), I recorded begging calls of chicks in both nest environments.

Chicks were removed from the nest and placed in a fake nest inside a box. The fake nest consists of an orange plastic bowl used as a nest platform in aviculture, tightly lined with nesting material from abandoned estrildid nests. Chicks were left in the fake nest with the box closed (or partially closed to allow air in and prevent overheating) for a few minutes to allow acclimation. To stimulate begging, the chick was tapped gently with forceps on the bill. To initially stimulate begging, tapping was more rapid than that which was subsequently used to sustain begging. Initial taps with the forceps would often lead to a slight and then complete opening of the mouth. Tapping inside the mouth would often elicit vocalizations. The hungrier the bird was, the less distinct these stages would be. Once the bird had started begging, the bird's beak would be gently tapped, approximately once every 3 seconds. For some birds this rate of tapping was too slow to maintain begging and the bird would go quiet. In such cases I increased the frequency of taps (but a note was made of this in the recording, and it is evident from videos).

Recording begging recordings in an artificial nest and stimulating begging manually has the disadvantage of a natural parent-offspring begging interaction is not recorded. However, I recorded chicks in a fake nest rather than inside the natural nest for the following reasons. First, host nest mates were often present alongside the transferred chick. This would make isolating which calls came from which chick very difficult if a microphone was strapped to the bird's nest and a natural parent-offspring encounter recorded. The calls from multiple chicks would overlap, making it difficult to extract call parameters from a single call. Second, estrildid host parents visit the nest only infrequently (around once per hour). This would make it logistically difficult to get recordings as microphones would have had to be strapped to nests for long periods of time before adequate material was obtained.

Recordings were made using an Audio-Technica ATR35S tie-clip microphone (2014 (part), 2015, 2016 and 2017 seasons) or a Sennheiser ME-66 shotgun microphone (2014 (part) season) held with a free hand approximately 3 cm away from the focal bird's mouth. If chicks did not beg even after 1 hour in the box, which most commonly occurred when chicks were older and apparently more distressed by the situation, I sometimes played back calls previously made of that species begging (not necessarily from that brood or at the exact same developmental stage). This would sometimes elicit begging calls. A note was made in the recording when this was done. Recordings were made for around 2 minutes or until at least 10 seconds of continuous begging were recorded, where possible.

Analysing the effect of host environment nestling begging calls

To examine the influence of host environment on begging call development in Pin-tailed Whydahs, I compared the begging calls of nestling Pin-tailed Whydahs in their natural Common Waxbill nests and having been transferred to Blue Waxbill nests.

Pin-tailed Whydah nestlings were found to make a range of call types over the course of development. Four distinct call types were identified by visual inspection of sonograms and by listening to the recordings (see Results section). All four call types could be identified both in Pin-tailed Whydahs developing in Common Waxbill nests and Pin-tailed Whydahs that had been transferred to Blue Waxbill nests.

As there were no call types exclusively produced by Pin-tailed Whydahs in their natural nests or by Pin-tailed Whydahs that had been transferred to Blue Waxbill nests, I analysed whether host environment influenced the stage in development at which each call type was made. To do this I quantified the spread of developmental stages of chicks making each call type and compared this between Pin-tailed Whydahs raised in Common and Blue Waxbill nests. Rather than coming up with completely new call types, it might instead be the case that Pin-tailed Whydahs use a certain call type over a during a larger portion of the developmental period in a Blue Waxbill nest compared to in their natural Common Waxbill nest. This could be because a certain call type is particularly effective at soliciting food from Blue Waxbill parents. I use chick tarsus length as a proxy for developmental stage. This was because for Pin-tailed Whydahs in their natural nests, the exact age in days of the chick was unknown. By contrast, chick tarsus was available for both Pin-tailed Whydahs in their natural

nests, and for Pin-tailed Whydahs transferred to Blue Waxbill nests. Chick tarsus was used over chick mass as chick tarsus is a more consistent measure which does not vary fluctuate with feeding and excretion like chick mass does.

I also examined whether within each call type, there were changes in call structure between host environments. For each call type, the following begging call parameters were extracted from each recording: minimum frequency, maximum frequency, centre frequency, peak frequency, frequency bandwidth, call duration, average entropy, and energy. These parameters are widely used in the literature on bird begging to characterise sounds (e.g. Anderson et al. 2009; Butchart et al. 2003; Langmore et al. 2008). For each recording, ten sequential call notes in a bout of begging were selected and the above parameters extracted. Call notes were not selected if they overlapped with interfering background noises, or if they were weak and incomplete calls.

The relationship between the call types was visualised using linear discriminant function analysis (DFA) with the R package MASS (Venables and Ripley 2002). Linear DFA takes explanatory variables (in this case call parameters) for each call type and creates a classification function based on a linear combination of those call parameters which best classifies each call to the correct type. Each call can be plotted on a two-dimensional graph and coloured by call type. This allows us to visualise how well the different call types can be grouped and also to see which call types are more similar to each other.

Two approaches were used to compare the structure of each call type between Pin-tailed Whydahs raised in Common Waxbill nests, and those raised in Blue Waxbill nests. First, a series of linear mixed models were constructed, with each call parameter as a separate response variable. Host environment and chick hunger were fitted as fixed effects and individual chick identity as a random effect. This mixed-effect framework allowed repeated measures of begging calls from the same individual chick to be included, while controlling for pseudoreplication. I examined whether host environment had a significant effect on the call parameter of interest. I controlled for multiple testing using a Bonferroni correction (Dunn 1961).

Second, I carried out a logistic regression analysis using the R package nnet (Venables and Ripley 2002). This approach allows all eight call parameters to be considered

at once, by testing for an effect of host environment on the centroid (multivariate mean) of these call parameters.

The effects of hunger on nestling begging calls

If Pin-tailed Whydahs raised in Blue Waxbill nests develop begging calls differently from those in Common Waxbill, it is important to establish whether this is a result of Pin-tailed Whydahs being fed less in a new host environment and so being hungrier, rather than due to the chick plastically altering its begging call to be more effective at soliciting investment from a different host species. If hungrier chicks alter their begging call structure or use different begging call types, this could account for any differences observed in Pin-tailed Whydah begging between the two host environments.

To examine whether certain call types were made by hungrier chicks, I carried out a mixed-effects model with crop score as an ordinal response variable, call type as a fixed effect and chick identity as a random effect, using the R package Ordinal (Christensen 2015). I compared the AIC of this model with one in which no fixed effects were fitted.

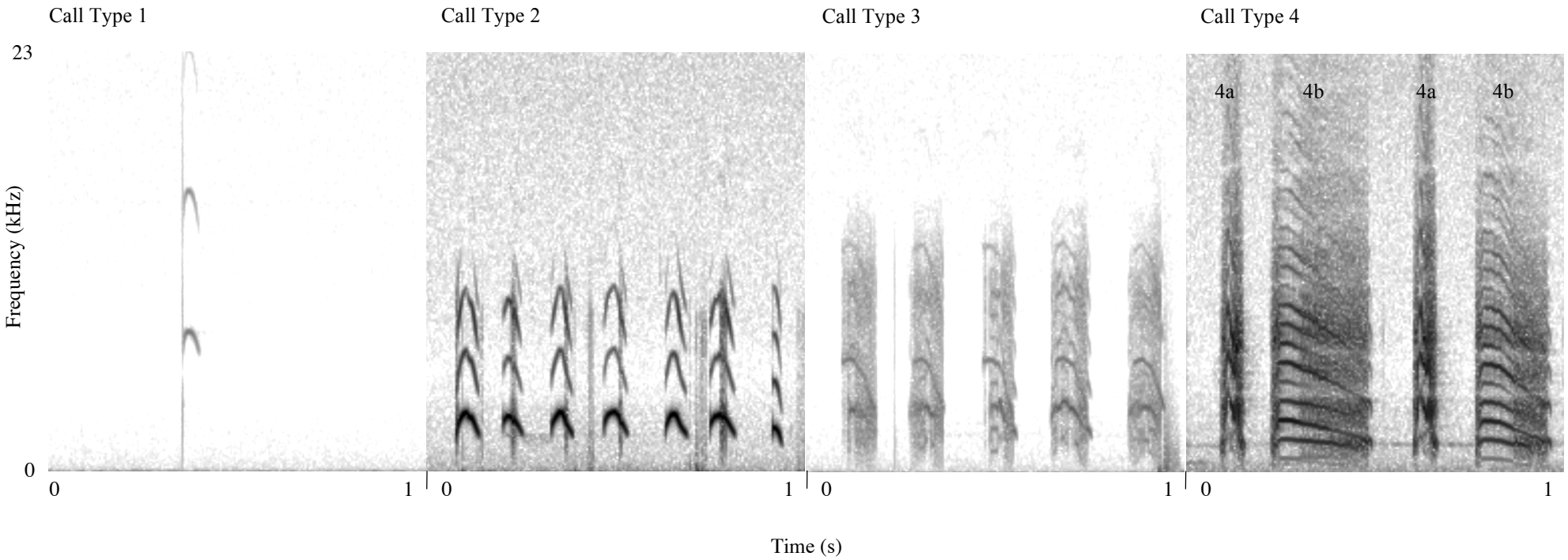


Figure 5.7. Example sonograms of the four qualitatively different types of Pin-tailed Whydah begging calls. Call type 4a is the shorter first note and call type 4b is the more drawn out, descending second note. Sonograms are all on the same scale: a 1 second time interval on x-axis and y-axis goes from 0–23kHz.

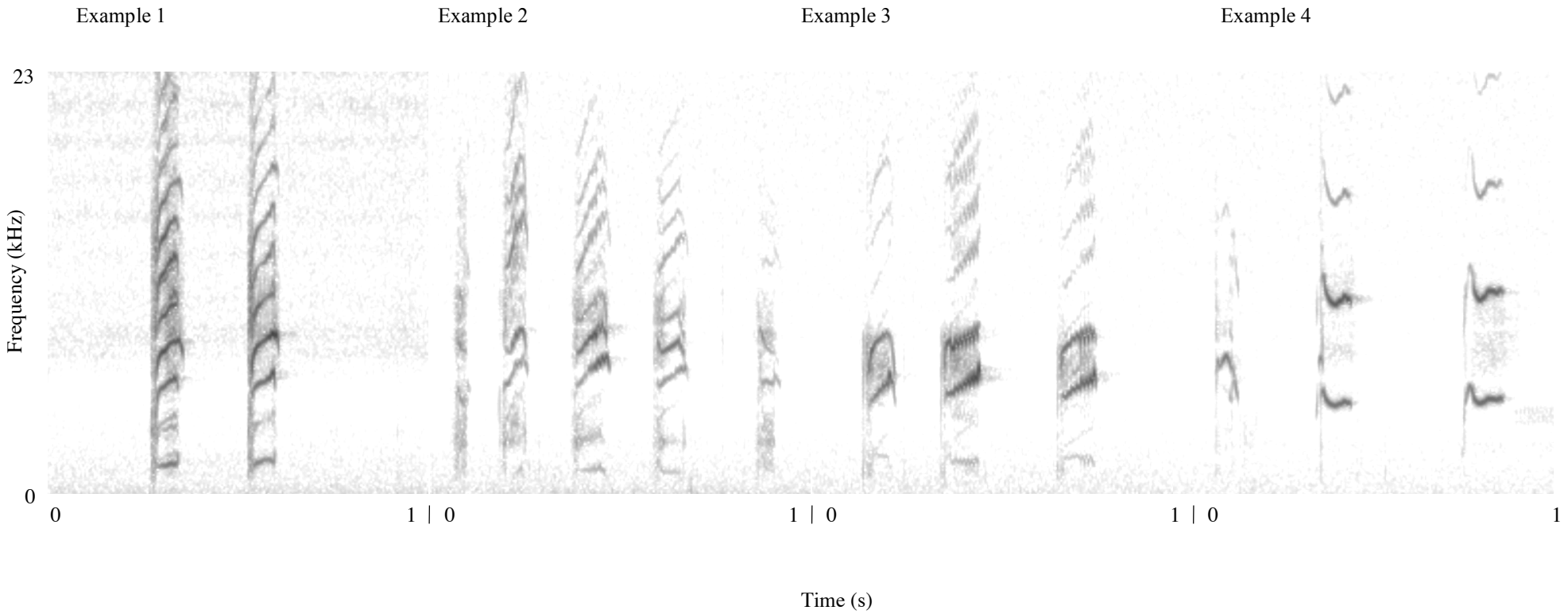


Figure 5.8. Example sonograms of begging calls from four different Blue Waxbill nestlings raised in their natal nests. All chicks are in mid to late development with their primaries having already irrupted from pin. Sonograms are all on the same scale: a 1 second time interval on x-axis for each example and the y-axis goes from 0–23kHz.

5.3 RESULTS

Hypothesis 1: Host parents reject heterospecific eggs

Of 81 foreign eggs placed in Blue Waxbill nests as part of the transfer experiments (35 Common Waxbill, 12 Pin-tailed Whydah, 2 Jameson's Firefinch and 32 Blue Waxbill eggs), none was rejected. Therefore, there is no evidence that Blue Waxbills reject eggs of other estrildid or *Vidua* species, or those laid by another Blue Waxbill female.

Hypothesis 2: Host parents reject heterospecific chicks

The foreign eggs that subsequently hatched (n = 81) and newly-hatched chicks (n = 17) transferred to Blue Waxbill nests comprised 38 Common Waxbill, 21 Pin-tailed Whydah, 4 Jameson's Firefinch and 35 Blue Waxbill eggs or chicks. None was confirmed to have been subsequently removed from the nest at the chick stage by the host parents while the chick was still alive. All removals of chicks from the nest seemed to happen after the chick had died in the nest and the body was removed. This was supported by observations at two nests in which a dead Common Waxbill chick was observed in the experimental nest in the morning, and was no longer present in the afternoon. The removal of the dead chick at one of these nests was captured on a trail camera. Therefore, there is no evidence of active chick rejection by Blue Waxbill parents of the chicks of other estrildid or *Vidua* species or those from another Blue Waxbill nest.

Hypothesis 3: Heterospecific chicks survive less well in a novel host environment than the novel host's own chicks do

Of the 94 transfers of Pin-tailed Whydahs (n = 21), Blue Waxbills (n = 35) and Common Waxbills (n = 38) to Blue Waxbill nests, selective death of the transferred chick occurred on 45 occasions. In the remaining 49 cases, the time to selective death of the chick was unknown (e.g. due to nest predation) or the chick fledged. In these situations, the data was right-censored.

The full model, containing the "presence/absence of host nestmates", "chick species" and "whether the chick had been transferred as an egg or a newly-hatched

chick" co-variates, explained the data significantly better than the null model (chi-squared = 35.7, df = 4, $p < 0.001$). This suggests that, among the predictor variables, at least one has a significant effect on survival. Dropping the "presence/absence of host nestmates" (chi-squared = 1.2, df = 1, $p > 0.2$) or the "whether the chick was transferred as an egg or newly-hatched chick" (chi-squared = 1.2, df = 1, $p > 0.2$) co-variates did not significantly decrease the explanatory power of the model. However, dropping the "chick species" did significantly reduce the explanatory power of the survival model (chi-squared = 34.4, df = 2, $p < 0.001$). This suggests that chick species has a significant effect on nestling survival in Blue Waxbill nests.

Comparing the survival of different chick species, Blue Waxbill nestlings transferred to other Blue Waxbill nests were found to survive significantly better than either Pin-tailed Whydah (survival analysis, $Z = 3.64$, $p < 0.001$) or Common Waxbill (survival analysis, $Z = 4.71$, $p < 10^{-5}$) nestlings transferred to Blue Waxbill nests. Survival of Pin-tailed Whydah nestlings in Blue Waxbill nests was not significantly different from that of Common Waxbill nestlings in Blue Waxbill nests (survival analysis, $Z = 1.25$, $p > 0.2$) (Figure 5.4).

Hypothesis 3.1: Host chicks are fed more by host parents than are heterospecific chicks

Median crop size during the first 7 days of life was found to differ significantly between the three species of transferred nestlings (Kruskal-Wallis, chi-squared = 14.23, df = 2, $p < 0.001$, Figure 5.5). Blue Waxbills had significantly higher median crop scores than Common Waxbill (Dunn post-hoc test, $Z = 3.12$, adjusted p-value < 0.01) and Pin-tailed Whydah (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, $Z = 0.597$, adjusted p-value = 1) (Figure 5.5).

When crop size was modelled as an ordinal response variable, a mixed-effects model with both chick species and number of nestmates as fixed effects (ordinal mixed-effect model, AIC = 525.04) was found to have a poorer fit than one with only chick species as a co-variate (ordinal mixed-effect model, AIC = 523.30), so number of nestmates was removed as a co-variate.

Crop scores differed significantly between transferred Blue Waxbill and Pin-tailed Whydah nestlings (ordinal mixed-effect model, $Z = 2.62$, $p < 0.01$) and between Blue Waxbill and Common Waxbill nestlings (ordinal mixed-effect model, $Z = 2.10$ $p < 0.05$). However, there was no significant difference between the crop scores of Pin-tailed Whydahs and Common Waxbills (ordinal mixed-effect model, $Z = 1.15$, $p > 0.2$). The result is robust whether the first six or eight days of development are considered (Table 5.1). However, if only the first four or five days of development are considered, the Blue Waxbill – Common Waxbill crop score comparison is not significant (Table 5.1).

The median crop size of chicks over the first 7 days of life was strongly associated with nestling survival (survival analysis, $Z = -3.667$, $p < 0.001$).

Table 5.1. Results of ordinal mixed-effect models comparing crop scores between each of the three transferred chick species over the first 4, 5, 6, 7 and 8 days of development. Significant differences in crop scores ($p < 0.05$) are in bold.

Species comparison	Days 0-4	Days 0-5	Days 0-6	Days 0-7	Days 0-8
Blue Waxbill vs. Pin-tailed Whydah	**2.12, p < 0.05**	**2.45, p < 0.05**	**2.35, p < 0.05**	**2.62, p < 0.01**	**2.53, p < 0.05**
Blue Waxbill vs. Common Waxbill	1.69, p > 0.05	1.60, p > 0.10	**2.07, p < 0.05**	**2.10, p < 0.05**	**2.16, p < 0.05**
Pin-tailed Whydah vs. Common Waxbill	0.953, p > 0.3	1.39, p > 0.15	0.845, p > 0.35	1.15, p > 0.20	0.968, p < 0.333

Hypothesis 3.2: Blue Waxbills feed their young a different diet than Common Waxbills do

Overview of taxonomic affinities of OTUs

DNA barcoding of nestling crop contents produced reads from 361 OTUs across all nestlings sampled, with a total of 909,620 reads. 900,217 of those reads (99.0%) mapped to the top 50 OTUs. For ease of analysis and taxonomic verification, only these top 50 OTUs were considered in the analysis.

49 of these 50 OTUs mapped to the grass family (Poaceae). The only exception was a single OTU that matched to the genus *Acalypha* in the sub-family Acalyphoideae of the Euphorbiaceae family. *A. villicaulis* and *A. allennii* are the most common species in the area but both are unlikely to produce much seed (Mike Bingham *in litt.*).

Of the 49 OTUs mapping to Poaceae, 41 of them match to grass species in the subfamily Panicoideae. The remaining 8 OTUs include 7 matches to the subfamily Chloridoideae and a single match to the subfamily Danthonioideae. No member of the Danthonieae is known from Zambia so this OTU likely refers to a species from another grass sub-family (Mike Bingham *in litt.*).

The 7 OTUs from the grass subfamily Chloridoideae matched to the genera *Chloris* (1), *Chloris/Eleusine* (1), *Orcuttia* (1), *Tuctoria* (2), *Tragus* (1) and *Muhlenbergia* (1). None of the genera *Orcuttia, Tuctoria* and *Muhlenbergia* occur in Zambia so these OTU likely refer to other genera in the Chloridoideae subfamily.

Of the 41 OTUs assigned to the grass subfamily Panicoideae, each matched to one or more of the following genera: *Andropogon, Arundinella, Brachiaria, Bothriochloa, Capillipedium, Cenchrus, Chrysopogon, Cymbopogon, Dichanthium, Digitaria, Echinochloa, Eriochloa, Hildaea, Hyparrhenia, Ichnanthus, Megathyrsus, Melinis, Panicum, Paspalum, Pennisetum, Pseudechinolaena, Saccharum, Schizachyrium, Setaria, Tricholaena, Urochloa* and *Zea.* Of these OTUs, the only genera that occur regularly in the Choma and are likely to produce seeds accessible and palatable to estrildid finches are: *Brachiaria, Capillipedium, Digitaria, Echinochloa, Eriochloa, Megathyrsus, Melinis, Panicum, Setaria, Urochloa* and *Zea* (Mike Bingham *in litt.*).

Comparing nestling diets between species at the sub-family level

10 of the 50 OTUs mapped to potentially more than one genus. Additionally, 14 of the remaining 40 OTUs that mapped to just a single genus, mapped to genera either not known to occur in Zambia or unlikely to produce palatable seed. This means that 24 of the top 50 OTUs have uncertain taxonomic identity at the genus level. By contrast, 49 of the top 50 OTUs can be confidently assigned taxonomic identity at the

sub-family level (the exception being the one OTU mapping to the Danthonioideae sub-family not known to occur in Zambia). Therefore, I decided to carry out the quantitative comparison of nestling diet between species at the sub-family rather than at the genus level.

Each OTU was assigned to one of the four sub-families identified: 1) Panicoideae, 2) Chloridoidae, 3) Acalyphoideae and 4) Danthonioideae. Whilst the OTUs assigned to Acalyphoideae and Danthonioideae do not necessarily belong to these sub-families specifically, I assume that they are correctly identified as not being members of either Panicoideae or Chloridoidae, and not being members of the same sub-family as one another.

For nestlings of all estrildid species, grasses from the subfamily Panicoideae dominate their diet, accounting for over 99% of reads in all seven species sampled (Table 5.2). However, despite their diet contents appearing to overlap extensively in Figure 5.6, there was a significant difference in the diet composition at the subfamily level between chicks in Common Waxbill and Blue Waxbill nests (pseudo $F = 5.347$, $p < 0.05$, Figure 5.6). The chicks in Common Waxbill nests are fed an even higher proportion of seeds from the subfamily Panicoideae (99.9%) than chicks in Blue Waxbill nests are (99.6%). However, despite this difference being statistically significant at the 5% level, it is unlikely to be biologically significant given how high the percentages are for chicks raised in both species nests. Therefore, at least at the subfamily level, the nestling diet of the estrildid finches occurring at the study site seem remarkably homogeneous.

Hypothesis 4: Parasite nestlings do not develop different begging calls in different host environments.

Diversity of begging call types produced by Pin-tailed Whydah nestlings

Before assessing whether Pin-tailed Whydah begging calls differed depending on host rearing environment, I documented the diversity of begging call types produced by Pin-tailed Whydahs.

Table 5.2. Median proportion of reads matched to each subfamily from DNA barcodes of nestling diet. Subfamily 4 relates to the single OTU matched to the subfamily Danthonioideae. However, this subfamily does not occur in Zambia so it is assumed to belong to another, unknown subfamily, here labelled "subfamily 4". Grasses from the subfamily Panicoideae dominate the nestling diet for all species of estrildid finch sampled.

Species	Number of nests	Panicoideae	Chloridoideae	Acalyphoideae	Subfamily 4
Blue Waxbill	20	0.996	0.00251	0	0.000610
Common Waxbill	9	0.999	0.000201	0	0.000301
Orange-winged Pytilia	4	1.00	0.000370	0	0
Melba Finch	3	0.993	0.00535	0	0.000582
Jameson's Firefinch	3	0.992	0.00250	0	0.00108
Bronze Mannikin	2	0.983	0.0165	0	0
Red-billed Firefinch	1	0.997	0.00136	0	0.00163
African Quailfinch	1	0.990	0.00734	0	0.00227

Pin-tailed Whydah nestlings produced a range of begging call types over the course of development in both Common Waxbill and Blue Waxbill nests. In total, I identified four call types from visual examination of sonograms and listening to recordings. These were: 1) short very high-pitched call given singly; 2) high-pitched calls given in quick succession; 3) a repeated mid-level call given by mid to old chicks; 4) a double call with two components, 4a and 4b. The two components usually come sequentially, with a short first note (call type 4a) immediately followed by a longer second note (call type 4b). I refer to call type 4 as the "we-chee", where "we" is 4a and "chee" is 4b. Example sonograms of each call type are given in Figure 5.7, and of Blue Waxbill calls in Figure 5.8.

Discriminant function analysis was used to plot the relationships of the different call types to one another (Figure 5.8). Linear discriminant 1 (LD1) explains

66.7% of the variation in these parameters, and LD2 explains a further 22.6% of the variation. Call duration made the largest contribution to the loadings of LD1 and LD2 (Table 5.3). Type 3 calls overlap on the graph of LD1 versus LD2 with call type 4a (Figure 5.8). This suggests that the two call types are very similar. The only distinction is that call type 4a is given before 4b in a quick two-note alternating pattern ("we-chee" call). By contrast, call type 3 is given singly or in rapid succession.

Table 5.3. Loadings of linear discriminant 1 (LD1) and 2 (LD2) used to differentiate call types produced by Pin-tailed Whydah nestlings. Call duration dominates the loadings of both LD1 and LD2.

	Average entropy	Frequency bandwidth	Call duration	Peak frequency	Centre frequency	Minimum frequency	Maximum frequency	Energy
LD1	-0.741	-1.92×10^{-5}	-26.5	-6.03×10^{-5}	2.46×10^{-4}	7.57×10^{-4}	1.08×10^{-5}	4.36×10^{-3}
LD2	0.123	3.19×10^{-5}	-27.5	-4.60×10^{-5}	-9.02×10^{-5}	-8.00×10^{-4}	9.49×10^{-8}	1.04×10^{-2}

Effects of host environment on the developmental stage at which Pin-tailed Whydah nestlings make different call types

The four different call types tended to be produced at different stages in chick development. When raised in their natural host's nest (Common Waxbill), Pin-tailed Whydahs make call type 1 during the first few days of development, start making call type 3 during mid-development, then incorporate call type 2 and, in late development, call type 4 (Figure 5.9).

However, the developmental stages at which call types were produced differed depending on whether the Pin-tailed Whydah was raised in a Common Waxbill or a Blue Waxbill nest (Figure 5.9). Using tarsus size as a proxy for developmental stage, call type 1 was produced by younger Pin-tailed Whydah nestlings when raised in Blue Waxbill nests compared to when raised in Common Waxbill nests (linear mixed model, $t = -3.09$, $p < 0.005$). Call type 2 was produced by younger Pin-tailed Whydahs when raised in Blue Waxbill nests, than when raised in Common Waxbill nests (linear mixed model, $t = -3.87$, $p < 0.001$). There was no significant difference in the tarsus lengths of Pin-tailed Whydah chicks producing call type 3 (linear mixed

model, t = -0.157, p > 0.8) or call type 4 (linear mixed model, t = -1.587, p >0.1) when raised in Blue Waxbill or Common Waxbill nests.

The effects of hunger on nestling begging calls

There was no evidence that Pin-tailed Whydah chicks were more likely to give any of their four call types when they had empty crops compared to when their crops were full, either when combining the data from Pin-tailed Whydah nestlings raised in Blue Waxbill and Common Waxbill nests (ordinal mixed-effects model, log-likelihood ratio = 5.539, p > 0.1), or when Pin-tailed Whydahs raised in Common Waxbill (ordinal mixed effects regression, log-likelihood ratio = 0.5681, p > 0.9) and Blue Waxbill (log-likelihood ratio = 6.3836, p > 0.05) nests were considered separately. However, the parameters of call type 2, 3, 4a and 4b did vary according to how full the crop was (Table 5.5). Only the parameters of call type 1 did not vary significantly with crop score.

Effects of host environment on the structure of each call type

For each begging call type produced by nestling Pin-tailed Whydahs, I analysed whether the call parameters differed depending on the host environment (Table 5.4). Call types 2 and 3 showed no significant difference in any of the eight call parameters depending on host environment. Frequency bandwidth was larger in Blue Waxbill-raised Pin-tailed Whydahs for call types 1, 4a and 4b. Call duration was longer in Blue Waxbill-raised Pin-tailed Whydahs for call type 4a. Peak frequency and centre frequency were higher in Blue Waxbill-raised Pin-tailed Whydahs for call type 1. Finally, minimum frequency was lower in call types 4a and 4b and maximum frequency higher in call types 1 and 4b for Blue Waxbill-raised Pin-tailed Whydahs (see Table 5.4 for statistics). Given that hunger was shown to influence some call parameters, the model was re-run with hunger as a co-variate. For call type 4a, host environment still influenced frequency bandwidth (chi-squared = 63.4, p < 0.001), call duration (chi-squared = 70.8, p < 0.001), minimum (chi-squared = 8.68, p < 0.01) and maximum (chi-squared = 56.1, p < 0.001) frequencies even when statistically controlling for hunger. Similarly, for call type 4b, host environment still had a significant effect on frequency bandwidth (chi-squared = 198, p <0.001), minimum (28.1, p < 0.001) and maximum frequencies (chi-squared = 182, p < 0.001) after statistically controlling for hunger.

Table 5.4. Effects of host rearing environment on Pin-tailed Whydah begging call parameters. Linear mixed models run with using maximum likelihood (REML = False) with chick individual identity as a random effect, and Pin-tailed Whydahs raised in Common Waxbill nests as the reference level. There are two host environments and so 1 degree of freedom. The chi-squared test statistic is given for each followed by p-value. Forty comparisons are made in this table, so the significance level has been adjusted to $p < 0.00125$ (= 0.05/40). Significant differences are in bold.

Call parameter	Call type 1	Call type 2	Call type 3	Call type 4a	Call type 4b
Average entropy	0.0981, $p > 0.7$	0.197, $p > 0.6$	0.615, $p > 0.4$	1.15, $p > 0.2$	2.85, $p > 0.05$
Frequency bandwidth	**14.331, $p < 0.001$**	7.31, $p > 0.0125$	0.223, $p > 0.6$	**13.1, $p < 0.001$**	**23.9, $p < 0.001$**
Call duration	3.76, $p > 0.05$	2.66, $p > 0.1$	0.562, $p > 0.4$	**10.9, $p < 0.001$**	0.108, $p > 0.7$
Peak frequency	**10.4, $p < 0.01$**	0.0028, $p > 0.9$	0.379, $p > 0.5$	0.303, $p > 0.5$	1.34, $p > 0.2$
Centre frequency	**14.5, $p < 0.001$**	1.08, $p > 0.2$	0.0904, $p > 0.7$	0.234, $p > 0.6$	0.851, $p > 0.3$
Minimum frequency	2.66, $p > 0.1$	0.0532, $p > 0.8$	1.06, $p > 0.3$	**47.6, $p < 0.001$**	**171, $p < 0.001$**
Maximum frequency	**32.3, $p < 0.001$**	8.11, $p > 0.00125$	0.154, $p > 0.6$	**10.7, $p < 0.00125$**	**20.7, $p < 0.001$**
Energy	0.139, $p > 0.710$	0.308, $p > 0.5$	5.87, $p > 0.01$	1.61, $p > 0.2$	1.52, $p > 0.2$

Table 5.5. Effects of crop size on parameters of each call type linear mixed models run with using maximum likelihood (REML = False) with chick identity as a random effect. There are four crop sizes (0, 1, 2 and 3) and therefore, 3 degrees of freedom. Here, Pin-tailed Whydahs raised in Common and Blue Waxbill nests are considered together. Chi-squared test statistic is given for each followed by p-value. Forty comparisons are made in this table, so the significance level has been adjusted to $p < 0.00125$ (= 0.05/40). Results for which crop score had a significant effect on the call parameter are in bold.

	Call type 1	Call type 2	Call type 3	Call type 4a	Call type 4b
Average entropy	3.52, $p > 0.3$	**42.5, $p < 0.001$**	**28.3, $p < 0.001$**	**72.2, $p < 0.001$**	**378, $p < 0.001$**
Frequency bandwidth	3.72, $p > 0.2$	14.6, $p > 0.00125$	10.3, $p > 0.01$	**130, $p < 0.001$**	**162, $p < 0.001$**
Call duration	5.05, $p > 0.1$	**72.6, $p < 0.001$**	**26.7, $p < 0.001$**	**20.2, $p < 0.001$**	**41.9, $p < 0.001$**
Peak frequency	8.27, $p > 0.01$	1.65, $p > 0.6$	**104, $p < 0.001$**	**16.0, $p < 0.00125$**	**353, $p < 0.001$**
Centre frequency	6.08, $p > 0.1$	2.74, $p > 0.4$	**95.2, $p < 0.001$**	**45.2, $p < 0.001$**	**114, $p < 0.001$**
Minimum frequency	5.09, $p > 0.1$	**65.3, $p < 0.001$**	**30.2, $p < 0.001$**	**62.0, $p < 0.001$**	**20.8, $p < 0.001$**
Maximum frequency	7.37, $p > 0.05$	4.82, $p > 0.1$	10.4, $p > 0.01$	**108, $p < 0.001$**	**152, $p < 0.001$**
Energy	3.74, $p > 0.2$	**808, $p < 0.001$**	**90.2, $p < 0.001$**	**81.6, $p < 0.001$**	**91.9, $p < 0.001$**

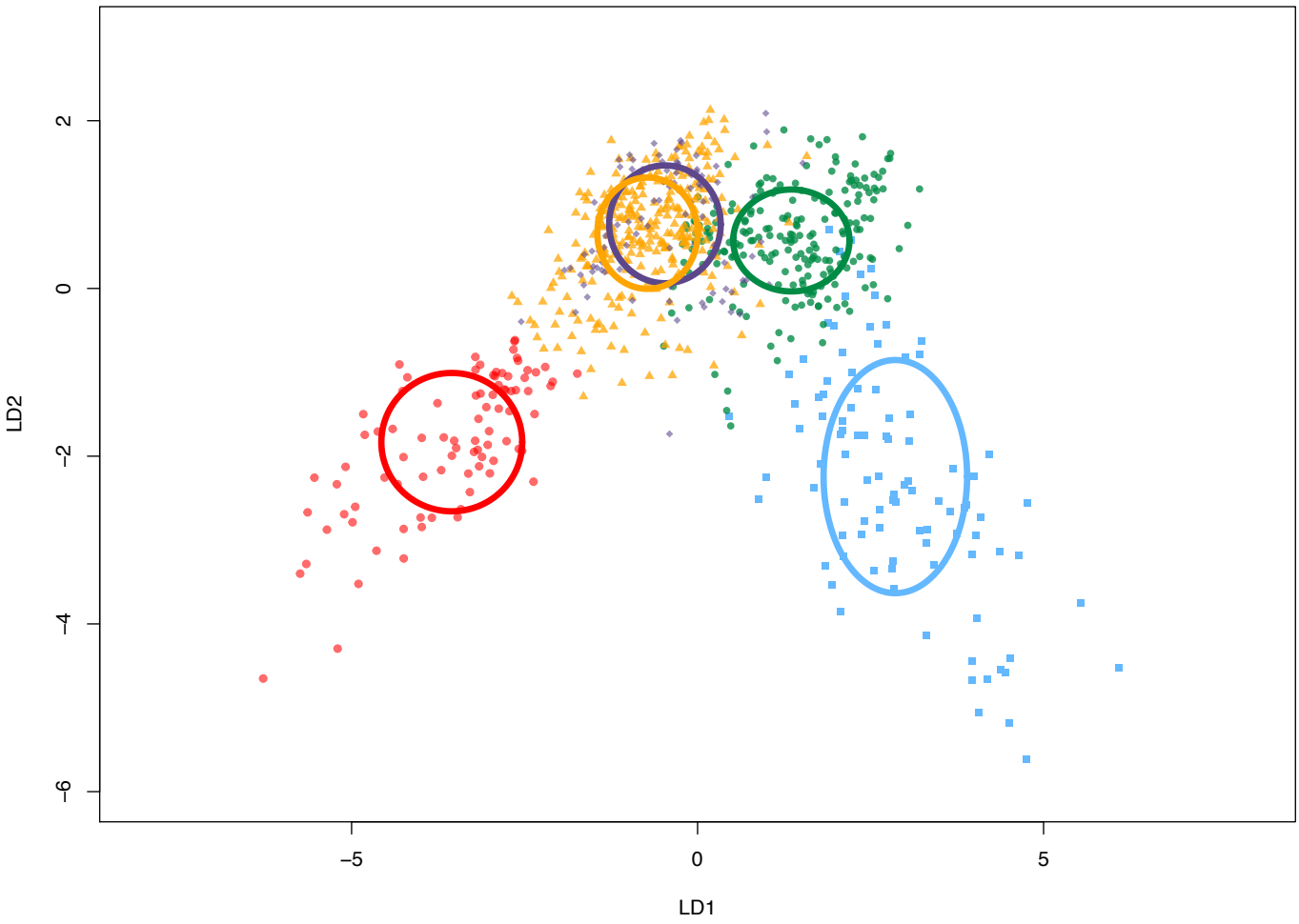


Figure 5.8. Discriminant function analysis of begging calls produced by Pin-tailed Whydah nestlings. This plot combines data from Pin-tailed Whydahs raised in Common Waxbill and Blue Waxbill nests. Discriminant function are based on the call parameters: minimum frequency, maximum frequency, centre frequency, peak frequency, frequency bandwidth, call duration, average entropy and energy. Linear discriminant 1 (LD1) explains 66.7% of the variation in these parameters, LD2 explains a further 22.6% of the variation. Call type 1 (light blue), call type 2 (green), call type 3 (orange), call type 4a (purple), call type 4b (red). Ellipses are centred on the mean values of LD1 and LD2 for that call type. Their widths on the x and y-axis about the mean are the standard deviations of LD1 and LD2 respectively.

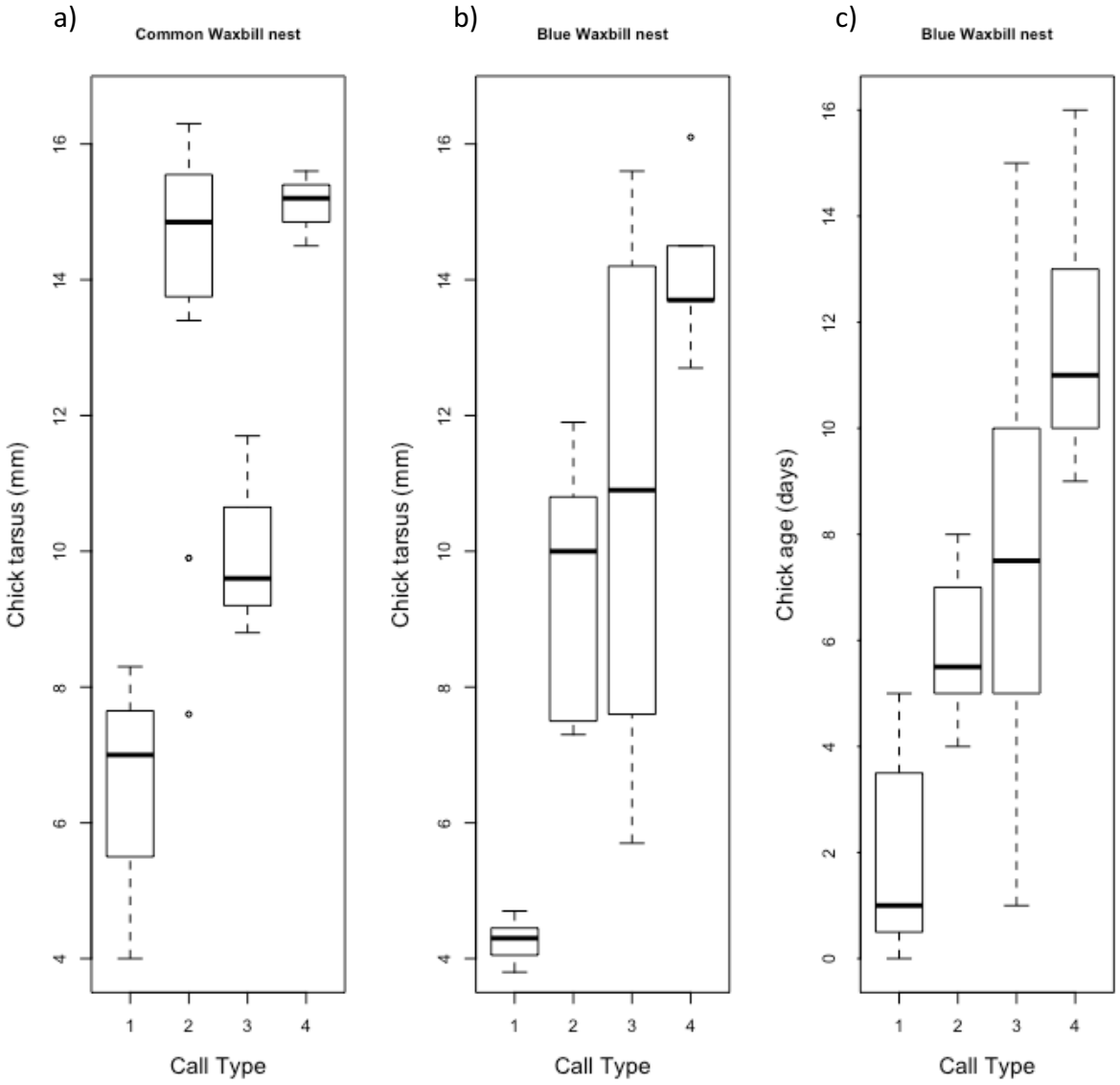


Figure 5.9. Pin-tailed Whydah nestlings produced different call types at different developmental stages and sizes. However, which call types are produced at which developmental stage, varied depending on the host environment; a) Pin-tailed Whydah nestlings raised in Common Waxbill nests; b) and c) Pin-tailed Whydah nestlings raised in Blue Waxbill nests. Tarsus length is used as a proxy for chick age in a) and b). Exact chick age data were available for Pin-tailed Whydahs raised in Blue Waxbill nests only, and this is shown in c) to demonstrate that conclusions are similar, as expected since tarsus size is a good proxy for chick age (Spearman's rank correlation = 0.932, df = 38, $p < 0.001$).

5.4 DISCUSSION

In this chapter I have examined the factors that could limit the colonisation of new hosts by avian brood parasites. Specifically, I focussed on the colonisation of a new host, the Blue Waxbill, by the Pin-tailed Whydah. Blue Waxbills are close relatives of the Pin-tailed Whydah's natural host, the Common Waxbill, but are typically not parasitised in nature. I experimentally simulated a host switching event by transferring Pin-tailed Whydah eggs and newly-hatched chicks to Blue Waxbill nests, and found that they survived less well in the Blue Waxbill nests than did cross-fostered Blue Waxbill eggs/chicks. Blue Waxbills did not show any evidence of egg or chick eviction behaviour, and the differences in survival seemed best explained by Blue Waxbill nestlings being fed more by the Blue Waxbill parents than were either Pin-tailed Whydah or Common Waxbill nestlings during the nestling period. The food that Blue Waxbill and Common Waxbill parents give their young is very similar, with grass seeds from a single subfamily (Panicoideae) accounting for more than 99.5% of the plant material in the crops of chicks raised in both environments. Pin-tailed Whydahs were found to produce a range of different call types, and all were produced in both host environments. However, there were differences in the developmental stage at which different call types were produced in the two environments, and some slight shifts in call structure for two of the four call types when raise by different host.

Absence of egg rejection behaviour by Blue Waxbill parents

That Blue Waxbill parents failed to reject eggs of other Blue Waxbills, Common Waxbills or Pin-tailed Whydahs is unsurprising. All members of the Estrildidae and Viduidae, with the notable exception of the Cuckoo Finch (*Anomalospiza imberbis*) (Spottiswoode and Stevens 2010; Spottiswoode and Stevens 2011), have plain white eggs differing only in dimensions. Therefore, unless there are cryptic differences in the ultraviolet spectrum (Starling et al. 2006) between host and parasite eggs, or host parents are able to use non-visual cues to detect foreign eggs, it is unlikely that they can distinguish between the two. However, Ostrich (*Struthio camelus*) females have been shown to distinguish between eggs laid by themselves and "parasitic" eggs laid by other Ostrich females despite their appearing identical to human eyes (Bertram 1979). It is still not known how they make the distinction.

As a follow-up experiment, it would be useful to see whether it is the close match in appearance of *Vidua* and Blue Waxbill eggs which prevent egg rejection, or whether Blue Waxbill simply show no egg rejection behaviour at all no matter how dissimilar the egg. To do this, one could put the eggs of Tawny-flanked Prinia (*Prinia subflava*), a common species at the study site with diverse and brightly coloured eggs similar in dimensions to Blue Waxbill (Spottiswoode and Stevens 2010; Spottiswoode and Stevens 2011), into Blue Waxbill nests. If Blue Waxbill parents showed no egg rejection behaviour whatsoever, we would expect that even these highly dissimilar eggs would be incubated.

Discrimination but no rejection of mismatching chicks by Blue Waxbill parents

Blue Waxbills were found to feed mismatching chicks (Pin-tailed Whydahs and Common Waxbills) less food than they fed experimentally transferred Blue Waxbill nestlings. The results suggest that Blue Waxbill parents specifically discriminated against mismatching chicks, rather than discriminating against chicks other than their own. This discrimination was apparent regardless of whether the transferred chick was raised alongside host young, or on its own. This suggests that Blue Waxbill parents have an internal template of what their own chicks should look or sound like, rather than only discriminating against the most odd-looking chick from amongst the current brood. Whether this internal template of chick appearance is innate or learned through interactions with their first brood is unclear. If it were learned, first-time breeders should be less discriminating against mismatching chicks than birds that have had several broods (Langmore et al. 2009; Lotem et al. 1995). . This variation in host experience with raising broods of their own young may account for why some transferred Pin-tailed Whydah and Common Waxbill chicks were not fed at all and did not survive for more a couple of days (generating the steep drop in the survival curves of Figure 5.4), whereas others were fed more food and survived for longer. If this were true, then it would suggest that any environmental factors increasing the proportion of first-time breeders in the population of a potential host would increase the likelihood of successful host colonisation by *Vidua*, as the average levels of discrimination against mismatching chicks would be lower. A good breeding season the previous year (e.g. due to good rains and high food abundance) could result in large numbers of first-time breeders making nests the following year, providing

relatively benign conditions for host switches. This also increases the likelihood of several *Vidua* simultaneously colonising the new host, making it more likely that the offspring will grow up to find a mate raised by the same host.

That estrildid parents discriminate against foreign chicks by feeding them less rather than ejecting them from the nest, allows us to understand how novel hosts can be colonised by *Vidua* species and how they can subsequently evolve such precise mimicry. If estrildid finches actively rejected any chick that did not look like its own, there would be no way that a parasite species could evolve from one fitness peak to another on an adaptive landscape. However, as estrildid finches only discriminate against odd chicks by feeding them less than they would a conspecific chick, it allows parasites a route to incrementally gain in fitness as their begging displays converge on those of the new host. This leads to the prediction that the more similar the begging displays between parasite and host, the greater the feeding rate by the host. If similarity in begging displays is the key factor in determining whether a parasite can successfully colonise a new estrildid host, it should be reflected in patterns of host colonisation observed more generally by *Vidua* – i.e. the "clade-limited" colonisation suggested by phylogenetic evidence (Sorenson et al. 2004). Specifically, this hypothesis predicts that species within a given clade should be more similar in begging call, mouth marking and head movements than they are to species in different clades (this prediction is tested for mouth markings in Chapter 6).

Dietary similarities between estrildid finch species

The nestling diets of all eight species of estrildid finch sampled at the study site in Zambia were very similar. The plant component of the diet of all species was dominated by grass seeds in the subfamily Panicoideae. There was a slight difference between Common Waxbill and Blue Waxbill diet in that Common Waxbill averaged an even higher proportion of grass seeds from this subfamily (99.9%) than did Blue Waxbills (99.6%). However, whilst statistically significant, it is unlikely that this difference is biologically significant.

DNA barcoding was not able to resolve differences in nestling diet at a finer taxonomic scale than the subfamily level, with many generic assignments being uncertain or mapping to genera not known to occur in the region. As more barcodes

for more species of Afrotropical grasses are included in reference databases, this resolution should increase. It is possible that there exist high levels of nutritional variability between grass species within the subfamily level which the current analysis is not capturing. Certainly, when Zambian farmers are choosing types of grass to graze cattle on they require specific species rather than any member of a subfamily (Bruce Danckwerts *pers. comm*).

The lack of niche differentiation in the diets of eight species of estrildid nestlings at the subfamily level is surprising. Traditional ecological theory suggests that closely-related species occurring in the same should have different foraging niches to co-exist without outcompeting one another (Hardin 1960). However, perhaps when the shared resource is as abundant as grass seeds are during the breeding season, it never becomes a limiting resource and so multiple species can subsist on overlapping diets.

The uniformity of nestling diets between estrildid finch species does, however, suggests that adaptation to novel diet is unlikely to be a major barrier in the colonisation of new hosts by species of *Vidua*. This is because adaptations allowing them to digest the diet of one host do make them well adapted for digesting the diet of a new host. Therefore, they are unlikely to encounter the trade-off between host-specific adaptation and being a generalist introduced at the start of this chapter.

Instead, it seems that the major barrier to colonising new hosts for parasites is not securing the right *type* of food from host parents, but rather ensuring they get fed the right *amount* of food. If this is true, then supplemental feeding experiments of transferred chicks should improve their survival. Furthermore, it also suggests that any conditions that increase the abundance of food in the area (such as good rains) is likely to increase the probability of simultaneous successful colonisations of new hosts.

Begging call plasticity in Pin-tailed Whydahs

Pin-tailed Whydahs were found to produce four different types of begging calls, all of which were made by Pin-tailed Whydahs raised in Common Waxbill nests and those

raised in Blue Waxbill nests. Therefore, being transferred to a new host environment did not lead to the development of novel begging call types in Pin-tailed Whydahs, as predicted by the framework established in Chapter 3. Additionally, none of the Pin-tailed Whydahs transferred to the nests of Blue Waxbills adopted the side-to-side head movements of that species. The inability of Pin-tailed Whydahs to secure as much provisioning from Blue Waxbill parents as did transferred Blue Waxbill chicks is likely due to the Pin-tailed Whydah chick having mismatching mouth markings, head movements and/or begging calls to those of a normal Blue Waxbill chick.

Although Pin-tailed Whydahs did not develop any new call types in the new host environment, the developmental stage at which each call type was employed did vary depending on which host was raising them. In Common Waxbill nests, Pin-tailed Whydahs produced call type 1 when very young, then call type 3 around the middle of development, and then call types 2 and 4 during mid to late development. By contrast, when raised in Blue Waxbill nests, Pin-tailed Whydahs produced call type 2 begging calls much earlier in development than when raised by their natural host. Interestingly, call type 2 is the one which, to my ears, sounds most like the high-pitched, short begging calls of a Blue Waxbill chick. It could be that the Pin-tailed Whydah chick is choosing, from its innate repertoire of call types, to use the call type which most resembles that of its host and is most effective at stimulating parents to feed them. This assertion could be tested first by quantitatively comparing each of the call types with that of Blue Waxbill nestlings to see whether call type 2 is quantitatively the one most similar to Blue Waxbill calls. Playback experiments of each Pin-tailed Whydah call type at Blue Waxbill nests could be used to see whether call type 2 elicits the greatest feeding rate from Blue Waxbill parents.

Within call types 1 and 4 but not call types 2 and 3, certain parameters did differ on between Pin-tailed Whydahs raised in Common Waxbill and Blue Waxbill nests (Table 5.4). For call type 1, the frequency bandwidth, peak frequency, centre frequency and maximum frequency were all greater in Pin-tailed Whydahs being raised in Blue Waxbill nests than those in their natural Common Waxbill nests. These differences are unlikely to be explained by Pin-tailed Whydahs in Common Waxbill nests being on average hungrier than those in Blue Waxbill nests. This is because hunger had no detectable effect on any of these call parameters for call type 1 (Table

5.5). It is unclear therefore, what has produced these changes. The overall shift could be described as a shift towards higher frequencies. Blue Waxbill chicks also make high frequency calls as young nestlings. Further work would need to quantitatively compare the shift in call type 1 to see whether it really does move towards the calls of Blue Waxbills. By contrast, the shifts in call type 4a and 4b are potentially explainable by Pin-tailed Whydah being hungrier in Blue Waxbill nests. Hunger was found to effect all measured call parameters in both call type 4a and 4b. However, host environment was still found to have a significant effect on these parameters even when hunger was included as a fixed effect in the model, suggesting that Pin-tailed Whydah nestlings may be shifting certain call parameters in response to interactions with hosts. Therefore, whilst there is no evidence of any large-scale shifts in call types produced in each environment, there may be slight changes in certain call parameters. Playback experiments would have to be done to see whether these shifts are adaptive and do better at soliciting investment from parents than natural calls.

The fact that *Vidua* mimic all three aspects of their natural host's begging display (Chapter 4) implies that convergence on host chick signals in all three of these channels leads to increased feeding from parents. When colonising a new host, however, it is not yet clear which of these three channels usually evolves to mimic hosts first. Given the potential for plasticity in the behavioural traits of begging calls and head movements, it might be expected that these traits would converge on the new host's the fastest. This could be tested by recording the begging calls of *Vidua* species that have naturally colonised new hosts recently. Prime candidates for this would be the population of Village Indigobird that has recently started parasitizing a new host, Brown Firefinch, near Livingstone, Zambia (Payne et al. 2002) or the population of Pin-tailed Whydah that seems to have recently colonised a population of Black-crowned Waxbill (*Estrilda nonnula*) in Cameroon (Lansverk et al. 2015).

Conclusions

To summarise, the findings of this chapter suggest that the key barrier to colonising new estrildid hosts for *Vidua* species is soliciting sufficient parental investment from the new host parents. That Pin-tailed Whydahs survived no better in the new host environment than did their natural host, Common Waxbill, implies that Pin-tailed Whydahs don't have any additional adaptations to aid survival in novel habitats. As

such, evolving the correct nestling appearance and begging calls seems essential to increase survival in foreign host environments. When the new host has vastly different mouth markings, begging calls and head movements from the ancestral host, this is likely to be a difficult task. A sudden abundance of resources, due to a good rainy season, could improve the probability of host colonisation both because parents might become less discriminating (due to the reduced costs of misallocation of food to unrelated nestlings) and because there are likely to be more naïve first-time breeders in the population (if the good conditions last for multiple years). This would give *Vidua* with mismatching mouth markings a better chance to survive in the foreign nest and subsequently a better chance of finding a mate who has been raised by the same host.

In this chapter I have only simulated the colonisation of a single new host. Future work could look at simulating the colonisation of other hosts with greater or lesser differences in begging display to the natural host. If mimicry of host begging displays is key to survival, *Vidua* nestlings would be expected survive worse in species with begging displays more dissimilar from that of their natural host. Mouth markings of estrildid finches show strong phylogenetic signal (Chapter 6), with species in the same clade having much more similar mouth markings than those in different clades. Further work could establish whether begging calls and head movements show similar levels of phylogenetic conservatism across the estrildid family tree.

Therefore, it seems that clade-limited colonisation (in which *Vidua* lineages tend to colonise new host species that are closely related to their ancestral host) is likely to be driven by combined influences of the phylogenetic inertia of host begging displays (Chapter 6), and the importance of mimetic begging displays in soliciting adequate feeding from host parents (this chapter). That host parents only discriminate against mismatching chicks by feeding them less, rather than ejecting them from the nest, likely provides parasites with a loophole explaining how *Vidua,* having colonised a host with reasonably similar begging displays, can evolve such remarkable mimicry over subsequent generations. It remains to be tested whether behavioural adaptations to hosts, which have the potential to develop plastically,

evolve faster than the more rigid morphological adaptations following host colonisation.

Chapter 6:
The evolution of nestling mouth markings in estrildid finches

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The evolution of nestling mouth markings in estrildid finches

6.1. INTRODUCTION

Grassfinches (family Estrildidae) have remarkably ornamented and diverse nestlings. This is unusual for birds, whose young are usually uniform and cryptically coloured. The drab appearance of most avian chicks is thought to camouflage defenceless young from predators (Kilner 2006b). However, ornamented nestlings have evolved in a few groups of birds: the terns (Sternidae), hoopoes (Upopidae), cuckoos (Cuculidae), rails (Rallidae), grebes (Podicipedidae) and passerines (all families in the Passeriformes) (Kilner 2006b). Conspicuous colours and patterns in the nestlings of these groups are largely restricted to the interior and lining of the mouth (except for the rails, Rallidae, and some members of the Tityridae whose nestlings can have bright red/orange plumages: (Krebs 2004; Londono et al. 2015). Most bird species whose nestlings have coloured mouths use different shades of orange, red and yellow to signal need to parents, while any spotting patterns are simple and restricted to the tongue (Kilner 2006b; Kilner and Davies 1998). By contrast, nestling estrildid finches have evolved a bewildering array of colours, patterns and structures both inside and along the gape of the mouth (Payne 2005b) (Figure 6.1). No other group of birds comes close to matching the diversity and complexity of appearance shown by these birds. Thus, the origins of this astonishing variation in nestling estrildid finches represent a perplexing mystery for evolutionary biologists to explain.

The Estrildidae are a family of 141 species of small passerines occurring across the Afrotropical, Oriental and Australasian regions as well as on many tropical Pacific islands. They occupy a wide variety of habitats from open grasslands, through savannahs to the interior of rainforests (Payne and Bonan 2017a). Estrildid nestlings show an interesting pattern of high between-species diversity in appearance but low within-species diversity, such that nestlings of most species have highly characteristic appearances. Visual traits varying between species include the mouth markings, skin

colour and the presence of natal down. Additionally, species differ in the begging calls and head movements made when soliciting investment from parents (see Chapters 4 and 5). The diversity of nestling phenotypes is in stark contrast to the uniformity of estrildid eggs, which are white, immaculate and vary between species only in size (Payne and Bonan 2017a). Comparable levels of nestling diversity and ornamentation to that seen in estrildids are found only in the couas of Madagascar (genus *Coua*, family Cuculidae) (Appert 1980). However, the *Coua* radiation contains only nine extant species. This results in lower overall diversity than estrildid finches, and fewer data points with which to test hypotheses.

In addition to occupying diverse habitats, estrildid finches also vary in whether they are brood hosts to indigobirds and whydahs (*Vidua* spp.) (Payne and Bonan 2017a; Sorenson et al. 2004). *Vidua* are host-specific brood parasites, most of whose nestlings accurately mimic the appearance of their host's (Payne and Bonan 2017b, and see Chapter 4 of this thesis). *Vidua* exclusively parasitise estrildid finches, mostly those in the genera *Lagonosticta* (firefinches), *Pytilia* (pytilias) and *Estrilda* (waxbills) (Table 6.1). There appears to have been strong selection for *Vidua* to mimic the mouth markings of their host (Payne et al. 2001; Schuetz 2005b, and see Chapter 5 of this thesis). Heterospecific chicks in foreign nests with mismatching begging displays have been shown to be fed less by parents and to survive or grow worse than conspecific, matching ones (Payne et al. 2001; Schuetz 2005b, and see Chapter 5 of this thesis). Parasitism by *Vidua* has a fitness cost on estrildid hosts because, while not evicting or killing nestmates, nestling *Vidua* compete with host young for parental resources (Payne and Payne 2002).

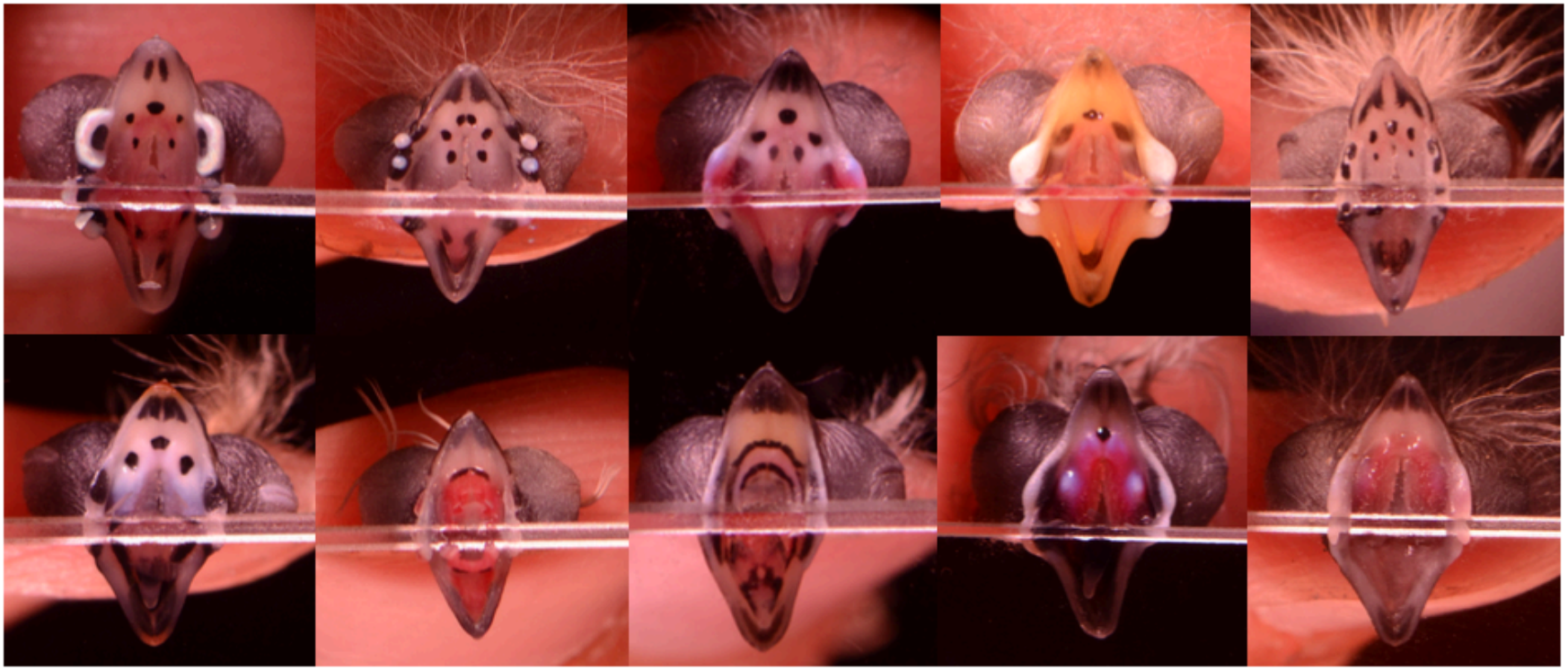

Figure 6.1. The diverse mouth markings of estrildid finches. Top row, left to right: Common Waxbill, African Quailfinch, Jameson's Firefinch, Red-billed Firefinch and Zebra Waxbill. Bottom row, left to right: Blue Waxbill, Locust Finch, Bronze Mannikin, Melba Finch, Orange-winged Pytilia. Images are arranged in order of decreasing ornamentation score. Common Waxbill and African Quailfinch have the highest ornamentation scores while Orange-winged Pytilia has the lowest.

To fully understand the radiation of nestling estrildid species, we must investigate both its origins and its subsequent diversification. The question of origins – namely, why such ornamentation arose at the base of this group of birds rather than any other – is difficult to answer. This is because ornamented chicks only had a single origin at the base of the family tree, making it challenging to know what the key factor was that led to its evolution in this group. One possibility is that, because estrildid finch parents feed young by regurgitating seed into their mouth, parents exert high levels of control over which chick gets fed how much. Chicks may therefore be selected to manipulate parental behaviour with elaborate signals which catch the parents' attention, rather than developing strategies which give the chick a physical competitive edge over its nest mates. Similar levels of control over food allocation are also shown in the rail family whose offspring are also ornamented (Krebs 2004; Lyon et al. 1994). However, finches of the subfamily Carduelinae in the family Fringillidae also primarily feed their young grass seeds through regurgitation, but their nestlings are not particularly ornamented (Collar et al. 2017). To investigate origins, a comparative study would have to be done across all bird groups in which ornamented young have arisen to see which ecological factors could have promoted its evolution. This chapter does not discuss the questions of origins in any more detail and instead examines the forces that could explain the extreme diversity of mouth markings observed between species within the estrildid finches.

We have more power to test hypotheses about mouth marking diversification than origin. This is because there exists huge diversity in mouth markings and overall appearance across the 141 species of estrildid finch. Using a comparative approach, we can reconstruct the evolution of these characters across the family and examine whether transitions in colour, pattern and overall complexity are correlated with certain ecological factors. We can also infer the rates at which these evolutionary shifts have occurred and again see whether ecological forces explain variation in rates of evolution.

Table 6.1. Known parasite-host associations between *Vidua* and estrildid finches (Payne 1973; Payne 1996; Payne 1998)

***Vidua* species**	**Host species**
Village Indigobird (*V. chalybeata*)	Red-billed Firefinch (*Lagonosticta senegala*)
Purple Indigobird (*V. purpurascens*)	Jameson's Firefinch (*L. rhodopareia*)
Dusky Indigobird (*V. funerea*)	African Firefinch (*L. rubricata*)
Baka Indigobird (*V. larvaticola*)	Black-faced Firefinch (*L. larvata*)
Wilson's Indigobird (*V. wilsoni*)	Bar-breasted Firefinch (*L. rufopicta*)
Jos Plateau Indigobird (*V. maryae*)	Rock Firefinch (*L. sanguinodorsalis*)
Cameroon Indigobird (*V. camerunensis*)	Black-bellied Firefinch (*L. rara*) Dybowski's Twinspot (*Euschistospiza dybowskii*) Brown Twinspot (*Clytospiza monteiri*)
Quailfinch Indigobird (*V. nigeriae*)	African Quailfinch (*Ortygospiza atricollis*)
Jambandu Indigobird (*V. raricola*)	Zebra Waxbill (*Amandava subflava*)
Zambezi Indigobird (*V. codringtoni*)	Red-throated Twinspot (*Hypargos niveoguttatus*)
Pin-tailed Whydah (*V. macroura*)	*Estrilda* waxbill species. Occasionally other species
Steel-blue Whydah (*V. hypocherina*)	Black-faced Waxbill (*Estrilda erythronotos*) Black-cheeked Waxbill (*Estrilda charmosyna*)
Long-tailed Paradise-Whydah (*V. paradiseae*)	Green-winged Pytilia (*Pytilia melba*)
Broad-tailed Paradise-Whydah (*V. obtusa*)	Orange-winged Pytilia (*P. afra*)
Exclamatory Paradise-Whydah (*V. interjecta*)	Red-winged Pytilia (*P. phoenicoptera*)
Togo Paradise-Whydah (*V. togoensis*)	Yellow-winged Pytilia (*P. hypogrammica*)
Sahel Paradise-Whydah (*V. orientalis*)	Green-winged Pytilia (*P. melba*)
Shaft-tailed Whydah (*V. regia*)	Violet-eared Waxbill (*Granatina granatina*)
Straw-tailed Whydah (*V. fischeri*)	Purple Grenadier (*Granatina ianthogaster*)

Hypotheses

In this chapter, I test several hypotheses about how ecological factors influence the evolution of nestling ornamentation. I do this by reconstructing the evolution of mouth markings across the estrildid family tree. Through reconstruction of ancestral character states and ecological conditions it is possible to examine whether shifts in ecology are correlated with evolutionary changes in characters. Hypotheses 1-3 relate to brood parasitism's influence on host mouth marking evolution and diversification. Hypotheses 4-7 relate to the influence of other ecological factors on estrildid mouth marking ornamentation levels.

Hypothesis 1: Brood parasitism selects for increased ornamentation in host nestlings

There are two trajectories by which parasitism could lead to increased ornamentation of host mouth markings. In the first scenario, *Vidua* converge on the host mouth markings and hosts are selected to evolve more elaborate, complex mouth marking 'signatures' that are more difficult for *Vidua* to mimic accurately (Payne 1977; Payne 2005b). This kind of co-evolution has been seen at the egg stage for several brood parasites (Brooke and Davies 1988; Spottiswoode and Stevens 2011; Spottiswoode and Stevens 2012). Here the host leads the way in the evolutionary arms race. In the second scenario, the parasite initially converges on the host and subsequently elaborates or exaggerates on the host's signal (Hauber and Kilner 2007). The parasitic chick can do this because, being unrelated to its nest mates, it is not constrained by kin-selected costs of outcompeting its nest mates for food, unlike host chicks. In response to the increased competition from parasites, hosts may be selected to converge on the exaggerated parasitic signal. Here, the parasite leads the way in the evolutionary arms race. Under both models, the result would be greater ornamentation in host compared to non-host estrildids.

In testing hypothesis 1, I also reconstruct the history of parasitism by *Vidua* in the family Estrildidae based on the patterns of parasitism shown by extant species. This will reveal the number of independent transitions from not being parasitised to being parasitised have occurred in the estrildid tree, which in turn will influence our power to detect an effect of parasitism on mouth marking evolution.

Hypothesis 2: Brood parasitism increases the rate of mouth marking evolution in hosts

Parasitism by *Vidua* may not necessarily select for increased ornamentation by hosts. Instead it could select for hosts to evolve dissimilar mouth markings from parasites. This would not necessarily produce a pattern of elevated levels of ornamentation in parasitised species; however, it should instead increase the rate of mouth marking evolution in parasitised lineages compared to unparasitised lineages.

Hypothesis 3: Parasitism by avian brood parasites increases rates of host speciation

Parasitism by *Vidua* may be expected to drive increases in speciation rates of host lineages, if parasitism drives faster rates of mouth marking evolution in hosts. This could result in the evolution of mouth marking differences between parasitised and unparasitised populations of a given estrildid species. These differences in nestling appearance may mean that any intergrades between the parasitised and unparasitised populations had intermediate phenotypes, and so had poor survival (Chapter 5). Such extrinsic post-zygotic isolation could then lead to pre-mating isolating factors eventually leading to the formation of separate species.

Hypothesis 4: Host mouth marking traits show strong phylogenetic signal

The pattern of host colonisation by *Vidua* is non-random (Sorenson et al. 2004). *Vidua* tend only to colonise new hosts that are in the same genus (or the same clade) as the ancestral host. This pattern has been termed "clade-limited colonisation" (Sorenson et al. 2004). One possible explanation for clade-limited colonisation is that species in a given clade have more similar mouth markings than those in different clades. This means that, when a *Vidua* lineage colonises a new host, it already has mouth markings that are close to those of its new host. Discrepancies in mouth markings between a foreign chick and its host have been shown to result in reduced feeding by parents and lower survival (Payne et al. 2001; Schuetz 2005b, Chapter 5 of this thesis). If similarity in mouth markings influences the pattern of clade-limited colonisation, we expect mouth markings to have a strong phylogenetic signal.

Hypothesis 5: Competition amongst siblings for access to parental care selects for increased ornamentation in nestlings

As competition for access to parental investment among nestmates increases, so selection for nestling adaptations to attract parental attention should increase (Godfray 1995). This

hypothesis therefore predicts that species with higher levels of sibling competition will show increased nestling ornamentation. Previous work has shown that nestling begging calls increase in loudness as the relatedness between nestmates decreases (Briskie et al. 1994). Lower relatedness among nestmates is expected to lead to increased sibling competition (Godfray 1991; Hamilton 1964). Therefore, Briskie's study supports the hypothesis that increased sibling competition results in more conspicuous nestling begging displays. Additionally, a comparative study on rails found that species with larger clutch sizes had more brightly ornamented chicks (Krebs 2004), consistent with a role for sibling competition in the evolution of nestling ornamentation.

While sibling competition is difficult to measure directly, clutch size has been used as a proxy in previous studies (Aviles et al. 2008; Kilner and Davies 1998; Soler and Aviles 2010). Higher clutch sizes mean that nestlings will, on average, have more nestmates to compete with, and so should experience selection for elevated ornamentation. However, the clutch size laid by any species of bird is likely an evolved response to, among other things, the availability of resources in the area. Therefore, if resource availability differs between two localities, the same clutch size might result in different levels of sibling competition. This is one of the reasons why clutch size is an imprecise proxy for sibling competition. An alternative measure of sibling competition would be the levels of brood reduction shown by the species (Soler and Aviles 2010). Brood reduction is thought to be a common feature of estrildid breeding biology and having been demonstrated both in the wild and captivity for several species (Hauber and Kilner 2007; Payne et al. 2001; Schuetz 2005a). However, these data are not available for enough species of estrildid finch to use in a comparative analysis, and so clutch size is used as a proxy instead.

Hypothesis 6: Nesting environments with low light levels select for increased nestling ornamentation

The light environment in which estrildid finches raise their young could influence the most effective design of an offspring-to-parent visual signal. Nestlings might be under selection to compensate for darker environments by producing more ornamented mouths (Aviles et al. 2008). Estrildid finch species all live in domed nests with a circular side entrance (Figure 6.2). Some species also have a long entrance tunnel (Tarboton 2011) (Figure 6.2). In the absence of direct data measuring the light environment inside the nests of each estrildid species, we can compare forest-living estrildid species with those living in more open

savanna and grassland habitats. Nestlings living in darker (forest) environments are expected to show elevated ornamentation compared to those living in lighter (savanna/grassland) environments.

Hypothesis 7: Nesting environments with higher predation levels select for increased nestling ornamentation

Bright and conspicuous nestlings may have evolved in an anti-predator context. For example, the bright orange hairs of nestling Cinerous Mourners (*Laniocera hypopyra*) have been hypothesised to be produced by Batesian mimicry of aposematic caterpillars and serve as an anti-predator warning signal (D'Horta et al. 2012; Londono et al. 2015). Additionally, the conspicuous colours and patterns of nestling *Coua* mouth markings have been speculated to be used to deter predators (Appert 1980).

If the bright colouration of nestling estrildid mouth markings has evolved to startle predators or signal distastefulness, nestlings would be predicted to be more ornamented in species that are raised in nests experiencing higher levels of predation. Predation pressure is difficult to measure directly for each species, but one proxy that can be used is nest height. Ground nests are generally subjected to higher levels of predation than nests that are elevated in a tree or bush (Martin 1993). Therefore, if the predator warning hypothesis is true, transitions to ground nesting should be correlated with increases in nestling ornamentation.

However, it is also possible that species subject to higher predation pressures are under selection to be less conspicuous, to reduce the likelihood of detection. For example, nestlings of species with higher predation rates have been shown to have evolved begging calls with higher frequency and lower amplitude than those subject to less predation, making the sound less easy for predators to locate (Briskie et al. 1999). Therefore, if the predator avoidance hypothesis is true, transitions to ground nesting should be correlated with decreases in nestling ornamentation.

Figure 6.2. (a) A Jameson's Firefinch (*Lagonosticta rhodopareia*) nest. This is the typical domed nest of most estrildid finch species. (b) A Common Waxbill (*Estrilda astrild*) nest showing the fake nest entrance on top and the real entrance, with the long entrance tunnel beneath. Long entrance tunnels evolved once in the common ancestor of all *Estrilda* waxbills and, independently, three other times in estrildid finches.

6.2 METHODS

Scoring estrildid nestling characters

Robert Payne attempted to quantify estrildid mouth markings and test some of these hypotheses in a comparative context (Payne 2005b). He divided the mouth of nestling finches into twenty characters and, for those species with available data, scored each based on published descriptions and his own photographs. This work produced extremely useful descriptions of the appearances of most estrildid finch species. However, there are several inconsistencies in his character scores as presented in Table 2 of that study. For example, Payne defines character 19 as the presence or absence of swelling on the palate. This character can take two states: yes (1) or no (0). This is specified explicitly in Table 1, p.6. However, scores of "2" are given for both *Aidemosyne modesta* and *Bathilda ruficauda*. Additionally, character 10 is defined as the "number of mediolateral spots" on the upper palate and can only take two values (0 or 2) according to Table 1. However, in Table 2, some species are given scores of 1, 3, 4 and 5. These values are not possible given how the character is defined in Table 1. There also exist several discrepancies between the written descriptions of a species and the character scores they receive in Table 2.

I therefore re-visited the descriptions and photographs of each estrildid species provided in Payne (2005) and in Handbook of Birds of the World online (Payne and Bonan 2017a) to verify the character scores. In situations where there was a discrepancy between the initial description/photograph and the score provided in Payne's Table 2, I noted this in my own character matrix and reported the value which agrees with the description/photograph. Of the 141 species of estrildid finch, thirty did not have information on mouth markings or photographs available. I scored the mouth markings for the remaining 111 species.

I decomposed estrildid mouths into 11 separate traits: 1) type of gape swelling on upper mandible, 2) type of gape swelling on lower mandible, 3) size of gape swelling, 4) number of medial palate spots, 5) number of lateral palate spots, 6) number of mediolateral palate spots, 7) number of palate bars, 8) whether palate bars are connected or not, 9) whether the palate is swollen or not, 10) the presence or absence of tongue markings, 11) whether there is a black ring running around the entire inner mouth. These are a subset of the 20 characters used by Payne (2005). The possible states for each gape character and the corresponding

ornamentation score are summarised in Table 2. The positions of some of these traits are shown in Figure 6.3. Examples of variations in gape swelling structure are shown in Figure 6.4. The frequencies of each character trait are shown in Figure 6.5.

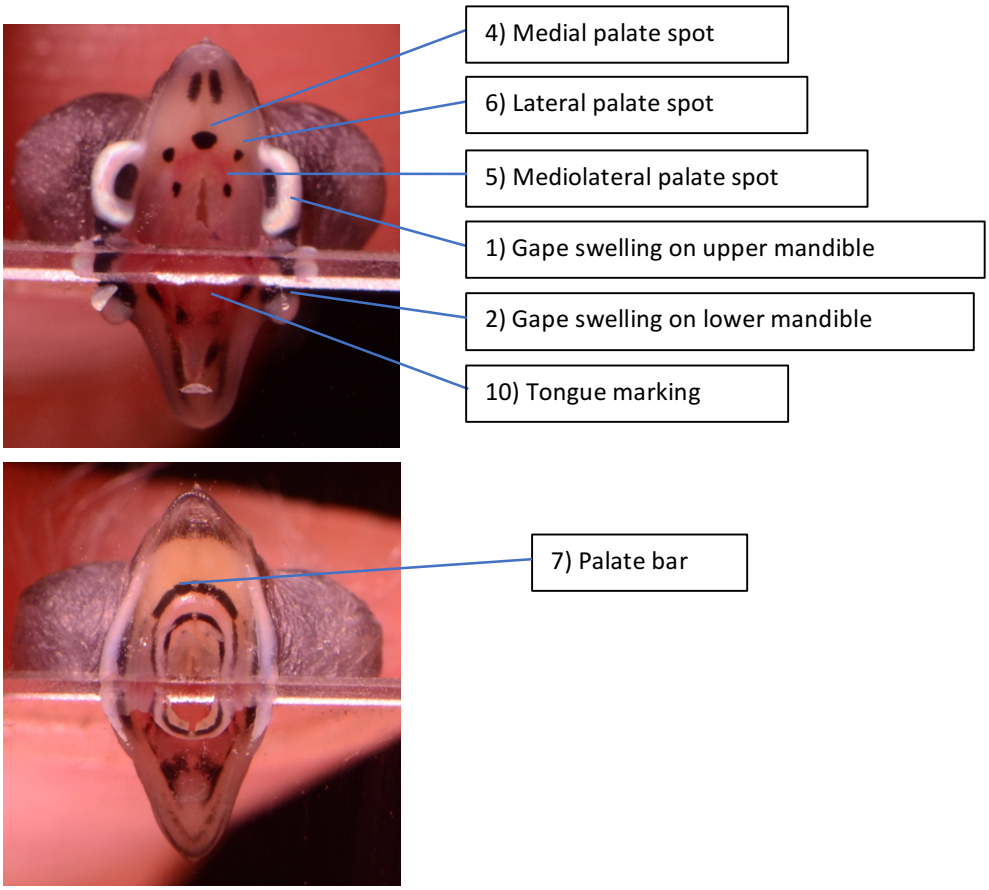

Figure 6.3. Nestling mouth marking characters of estrildid finches. The number next to each character relates to the trait number in in Table 6.2. On top is a Common Waxbill (*Estrilda astrild*) and, below, a Bronze Mannikin (*Spermestes cucullatus*) nestling. The mouth markings of estrildid finches are always approximately bilaterally symmetrical, so the spots on the chick's left hand side are mirrored on their right-hand side.

To allow the complex multi-dimensional nature of estrildid mouth markings to be quantified and visualised more easily, I used two approaches that collapsed the mouth markings into a single metric. The first approach was an "ornamentation index", calculated by summing across the conspicuous mouth marking characters (see Table 6.2). The ornamentation index was calculated by summing across the scores for 11 gape characteristics (see Table 6.2). For each gape character, a maximum score of 1 was available. Summed

across the 11 traits, therefore, the maximum ornamentation score was 11. The ornamentation index is calculated in a similar way to Payne's (2005) "index d". However, it differs in that, first, Payne's index did not include tongue markings. I included tongue markings because they are found widely across avian nestlings and because the precise pattern of marking (band, ring, spots or none) varies between estrildid species. Second, Payne scored all forms of upper and lower gape swelling as a "1", whereas I only score "arches" and "balls" as a "1" (see Table 6.2). Therefore, according to Payne's scoring system, every estrildid finch is given a "1" for both their gape swelling on the upper and the lower mandibles. This makes it an uninformative trait to understand differences in ornamentation level between species. By only scoring more elaborate types of gape swelling as a "1" and the rest as "0", the characters become more informative.

The second approach was "mouth marking appearance" index, calculated using multiple Components Analysis (MCA). MCA is the equivalent of Principal Components Analysis but applied to categorical data. All the mouth marking character scores (besides the ornamentation index) are categorical and therefore MCA must be used to reduce the dimensionality of the data. This approach also allows us to see which mouth marking characters are correlated with each other.

MCA was initially carried out on the 102 species of estrildid finch that had full mouth marking data, for the following traits: 1) number of medial palate spots; 2) number of lateral palate spots; 3) number of mediolateral palate spots; 4) presence of a palate bar; 5) presence of a black ring around the mouth; 6) presence of connected palate spots; 7) presence of tongue markings; 8) presence of palate swelling; 9) type of swelling on upper gape; 10) type of swelling on lower gape; 11) size of gape swellings. Palate bars (character 4) and tongue marks (character 7) were treated as binary, presence/absence, traits for the MCA rather than as categorical variables with many levels. This was done to simplify the analysis and help increase the information content of the first dimension of the MCA. Importantly, this index characterises variation in markings, and is not necessarily associated with degree of ornamentation. MCA was carried out using the *MCA* function from the R package FactoMineR (Lê et al. 2008), and figures were drawn using the R package factoextra (Kassambara and Mundt 2017).

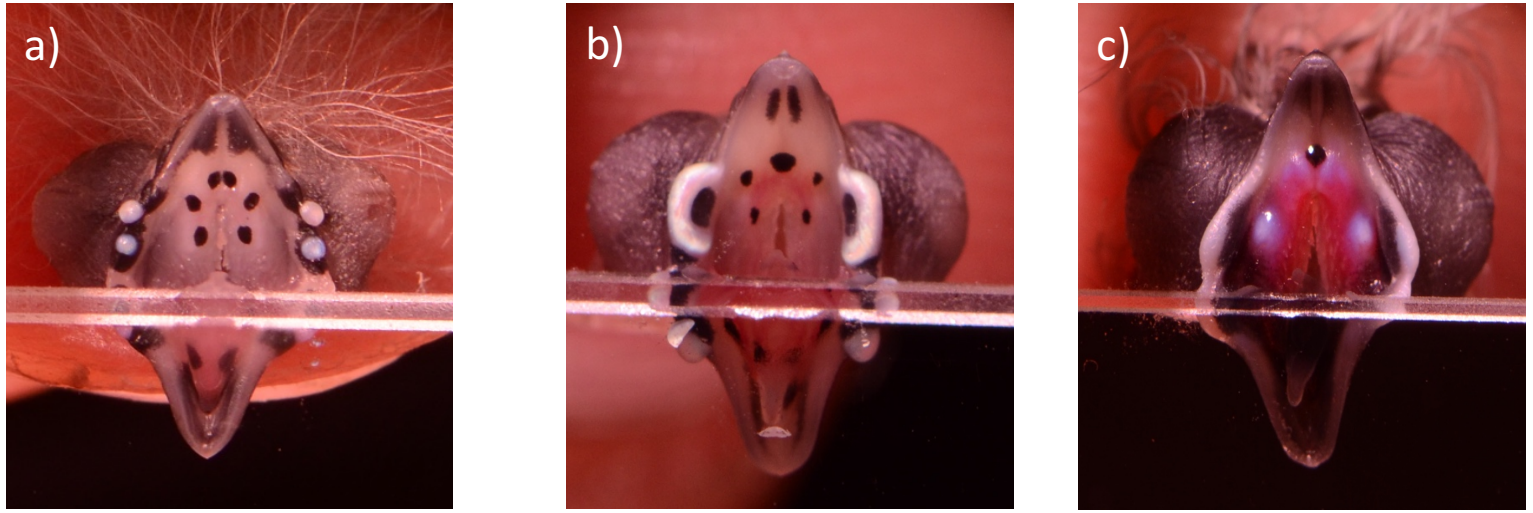

Figure 6.4. Variation in gape swelling structure between estrildid finch species. (a) African Quailfinch (*Ortygospiza atricollis*) showing "papillae" on the upper gape, (b) Common Waxbill (*Estrilda astrild*) showing "arcs" on the upper gape, (c) Melba Finch (*Pytlia melba*) showing a "ridge" along the upper gape.

Table 6.2. Scoring system for ornamentation index of nestling mouth markings**.**

Character	Levels	Ornamentation score
1) Type of gape swelling upper	Ridge/flange Ball Arc	0 1 1
2) Type of gape swelling lower	Ridge/flange Ball Arc	0 1 1
3) Size of gape swelling	Small Medium Large	0.33 0.66 1
4) Number of medial palate spots	0 1 2	0 1 1
5) Number of lateral palate spots	0 2	0 1
6) Number of mediolateral palate spots	0 2	0 1
7) Number of palate bars	0 1 2	0 1 1
8) Palate spots connected	No Yes	0 1
9) Palate swollen	No Yes	0 1
10) Presence of tongue markings	No Yes	0 1
11) Black ring around inner mouth	No Yes	0 1

Ecological characters

Information on estrildid clutch size, habitat, nest height and whether or not they are parasitised by *Vidua* was obtained from the Handbook of Birds of the World Alive (Payne and Bonan 2017a). For clutch size, the midpoint of the minimum and maximum reported clutch size for each species was used. Habitat was divided into two broad classes – closed, low light environments (forest and thicket) and open environments with more light (savannah and grassland). Nest height was also divided into two broad categories: ground (or within 1 m of the ground) and tree (> 1 m above the ground). Minimum and maximum recorded nest heights were also recorded, to get a continuous measure of nest location; however, this information was missing for 69 of the 141 species and so was not used in the analysis.

Evolutionary relationships

Payne's 2005 study was hindered by the lack of a comprehensive phylogeny of estrildid finches available at the time, meaning he was unable to examine the evolution of these character traits in a phylogenetically-controlled framework. Instead, he had to use an indirect approach to try to estimate the effect of phylogeny. He did this by constructing an index comparing the number of character differences between a given taxon and its inferred sister species (his "index a1"), and compared that value with other the number of differences between that taxon and a sympatric estrildid in the same clade (his "index a2"). If differences between species' mouth markings are largely explained by their phylogenetic relationships, one might expect index a1 to generally be smaller than index a2. This approach also made it impossible for Payne to reconstruct ancestral character states. Payne acknowledges the limitations of his approach, saying that a more direct approach is "deferred until a more comprehensive estimate of phylogenetic relationships (between estrildid finches) is available." (Payne 2005b, p.7).

For the present study, a more comprehensive phylogeny of estrildid finch relationships was available (Figure 6.6), produced by Professor Michael Sorenson at Boston University. The tree was generated using mitochondrial DNA and expands on previously published phylogenies of estrildid finches (Sorenson et al. 2004; Sorenson et al. 2003). It includes data from 254 estrildid finch samples representing 134 species, plus 33 outgroup samples. Sequences from two mtDNA regions (1) the NADH dehydrogenase subunit 2 (ND2) gene and portions of the flanking tRNAs, comprising 1088 base pairs in estrildids; and (2) a region comprising most of the ND6 gene, tRNA-Glu, and the 5' half of the control

region, comprising 1087 to 1123 base pairs in estrildids. Tree construction methods followed Gomes et al. (2016).

The resulting phylogeny was calibrated using the estimate of 15.69 million years for the divergence between Estrildidae and Viduidae (Gibb et al. 2015), corresponding to 11.71 million years for the common ancestor of estrildid finches (Gomes et al. 2016).

Reconstructing ancestral states

Ancestral states for continuous traits were reconstructed using the *fastAnc* function from the R package phytools (Revell 2012). *fastAnc* calculates maximum likelihood ancestral states for a continuous trait by taking advantage of the fact that the state computed for the root node of a tree in Felsenstein's contrasts algorithm (Felsenstein 1985) is also the maximum likelihood estimate of the root node. Therefore, the function re-roots the tree at all internal nodes and computes the contrasts state at the root each time (Revell 2012). Ancestral states for categorical traits were reconstructed using the function *ace* from the R package caper (Orme et al. 2013). This function carries out a maximum likelihood estimation of the ancestral character states. The states are computed with a joint estimation procedure using a protocol similar to that described in Pupko et al. (2000).

Calculating phylogenetic signal

Phylogenetic signal is defined as "tendency for related species to resemble each other more than they resemble species drawn at random from the tree" (Blomberg and Garland 2002). For continuous traits, phylogenetic signal can be quantified using Blomberg's K parameter (Blomberg et al. 2003) or Pagel's λ (Pagel 1999). If $K = 1$, it suggests that the traits have been evolving across the phylogeny according to a Brownian motion (random walk) model of evolution. If $K < 1$, it suggests that species are less similar than expected based on the phylogeny. If $K > 1$, it suggests greater similarity than expected based on phylogeny. Therefore, values of $K \geq 1$ indicate phylogenetic signal in the trait of interest. For estrildid mouth marking traits, K was calculated using the function *phylosignal* from the R package picante (Kembel et al. 2010).

Pagel's λ values can vary from between 0 and 1, where 0 indicates no phylogenetic signal and 1 indicates strong phylogenetic signal. λ is a measure of how the phylogenetic tree must be transformed to represent the way in which the trait of interest is evolving. If $\lambda = 0$,

the tree is transformed to a "star phylogeny" with all branches radiating out from a single point and the trait having no phylogenetic structure. If $\lambda = 1$, the traits are consistent with a Brownian motion model of evolution over the phylogeny. For estrildid mouth marking traits, λ was calculated using the *pgls* function from the R package caper (Orme et al. 2013). The λ value is calculated using maximum likelihood and the AIC compared to one in which λ is constrained to be 0 or 1.

For binary traits, the D statistic of Fritz and Purvis (2010) , was used to estimate phylogenetic signal. The statistic is based on the sum of the differences in trait value between sister clades scaled by the sum expected if the trait were evolving in a phylogenetically random manner. Therefore, if $D = 1$ the binary trait has a phylogenetically random distribution across the phylogeny tips. $D = 0$ implies the trait has evolved by Brownian motion across the phylogeny. Values of D below 0 suggest even stronger phylogenetic clustering than expected by Brownian motion. For estrildid mouth marking traits, D was calculated using the function *phylo.d* from the R package caper (Orme et al. 2013).

Stand-alone methods for estimating phylogenetic signal in categorical traits with more than 2 levels do not exist in standard packages, and so phylogenetic signal was not estimated for traits of this type. To obtain measures of phylogenetic signal for ordered categorical variables (gape swelling size, number of mediolateral spots), they were treated as continuous variables and K and λ values calculated.

Phylogenetic generalised least squares regression (PGLS)

Ecological hypotheses (1, 5, 6 and 7) were tested in a single model using the *pgls* function from the package "caper" (Orme et al. 2013) in which ornamentation index was modelled as a function of the species' parasitism status, clutch size, nesting habitat and whether it nested on the ground or in a tree. *pgls* fits a linear model that controls for phylogenetic non-independence between data points. The analysis was carried out on the 93 estrildid species with complete information on ornamentation index and the four ecological factors. After the model was fitted, normality of residuals was checked by visual inspection of a qq-plot, and homogeneity of residuals checked by visual inspection of a plot of fitted values versus residuals.

Rates of evolutionary change

The impact of parasitism on the rates of mouth marking evolution was tested using Ornstein-Uhlenbeck models in the package "OUwie" (Beaulieu and O'Meara 2016), with mouth marking appearance MCA dimension 1 as the response variable. Ornstein-Uhlenbeck models are more general form of Brownian Motion models. Whereas Brownian Motion models assume a random walk over character space, Ornstein-Uhlenbeck models allow for evolution to move around some optimum trait value (θ) at a certain rate (σ) and with a certain "pull" (α) back towards that optimal value. It is sometimes termed a "rubber band" model because the strength of the pull towards the optimum increases in proportion to the distance of the current trait value from the optimum. When there is no pull (α=0), the OU model simplifies to a BM model. The OUwie package allows users to reconstruct a "state" variable over the tree (in this case parasitised or not parasitised) and then compare the likelihoods of models in which each of the three parameters (θ, σ, α) can have different values for each state or is constrained to have the same value for both states. By comparing the likelihood of a model in which the rate variable, σ, can vary between parasitised and non-parasitised lineages with one where it is constrained, we can test the hypothesis that parasitism is linked to different rates of mouth marking evolution.

Diversification rates

The influence of parasitism on rates of diversification in estrildid lineages was carried out using the R package diversitree (FitzJohn 2012). Diversitree compares the log-likelihood of a model in which speciation rates can differ depending on a species' parasitism status, with one in which speciation rates are constrained to be equal across character states. If the former model explains the tree significantly better that the latter (having penalised the model for including an extra parameter) then there is evidence that brood parasitism is associated with different speciation rates in the host family's phylogeny.

6.4 RESULTS

Summarising the diversity of estrildid mouth markings:
Multiple Correspondence Analysis (MCA)

MCA of estrildid mouth markings was initially carried out on the 102 estrildid species with complete information on the 11 mouth markings specified in the methods. Dimension 1 explained 25.91% of the variation, and Dimension 2 explained a further 13.77%.

When Dimension 1 and Dimension 2 were plotted against each other, there were five species that lay far from the PC1 axis of variation. These were *Aidemosyne modesta*, *Emblema pictum*, *Stagonopleura guttata*, *Zonaeginthus bellus* and *Z. oculatus*. All five of these species occur outside of Africa and are therefore not parasitised, and nor have their lineages ever been parasitised if the ancestral reconstructions of parasitism are accurate (see Figure 6.10). *Z. bellus, Z. oculatus* and *S. guttata* are outliers because they are the only taxa both with palate spots connected and a palate swelling. *A. modesta* is also one of the few species with palate swelling, while *E. pictum* has the unusual combination of a black ring around the mouth, a palate bar and two mediolateral spots. To generate a more informative Dimension 1 of the MCA, and because none of these taxa are important for the analysis of the influence of parasitism on the rate of mouth marking evolution, these five species were removed from the analysis. Additionally, there are three mouth marking traits only occurring in a few Australian representatives of the estrildid family. These are connected palate spots, a black ring around the mouth and palate swelling. None of these traits are present in any African estrildid finches. To simplify the analysis and increase the explanatory power of Dimension 1, these three traits were excluded from the MCA. With these five species removed, a MCA was carried out on the remaining 97 species of estrildid finch using the remaining eight traits. Dimension 1 explained 31.67% of the variation and Dimension 2 explained a further 15.59%.

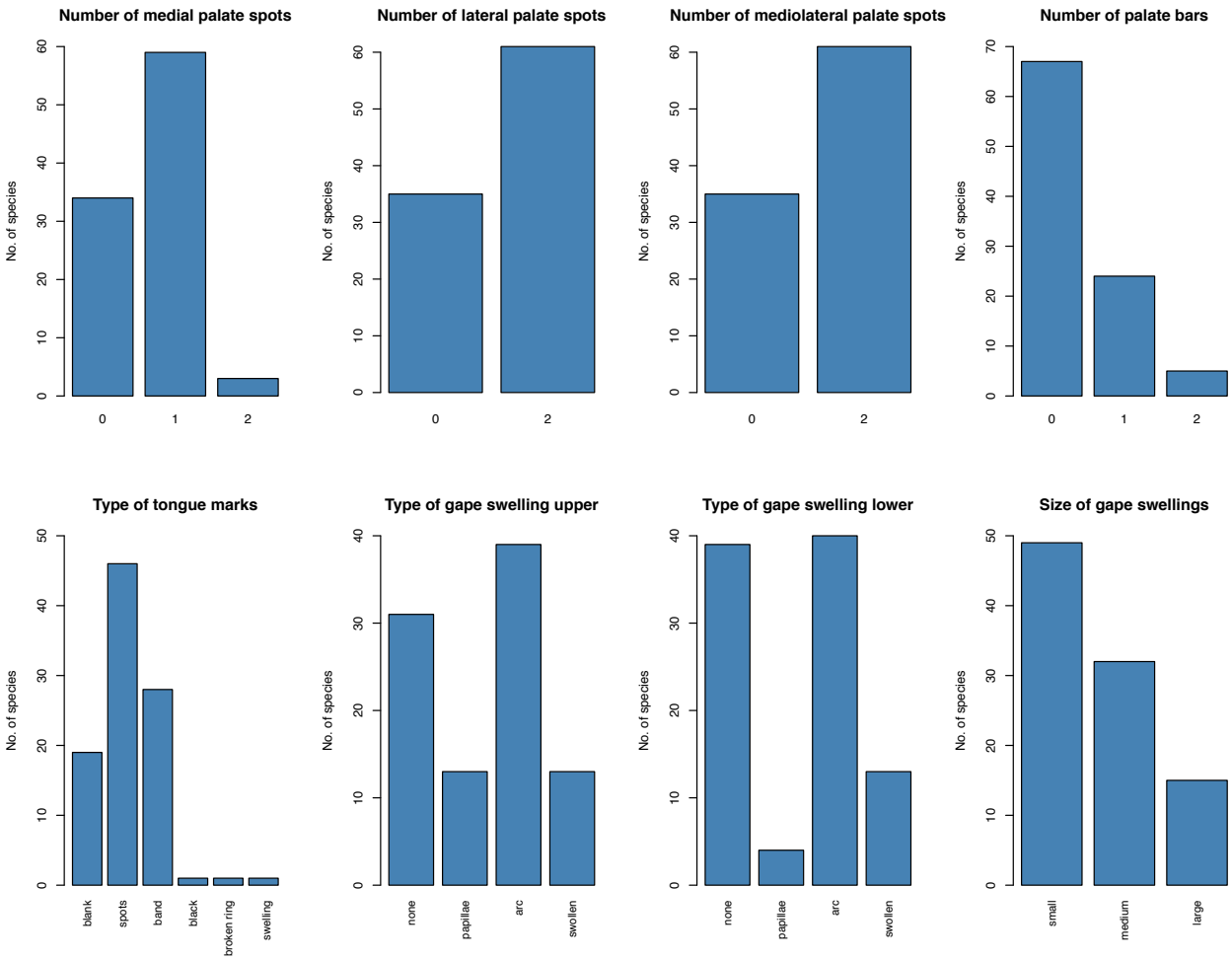

Figure 6.5. The frequencies of different mouth marking characters across all estrildid finch species

The mouth marking variables most correlated with dimension 1 are: number of medial palate spots ($R^2 = 0.848$, $p < 0.001$), number of lateral palate spots ($R^2 = 0.813$, $p < 0.001$) and the presence of a palate bar ($R^2 = 0.742$, $p < 0.001$). Thus, Dimension 1 describes an axis where species with large positive values have a palate bar, lack medial or lateral palate spots, and have a small swollen gape on both the upper and lower mouth. Examples of such species include the *Spermestes* and *Lonchura* mannikins, Locustfinch (*Paludipasser locustella*), Grey-headed Silverbill (*Odontospiza caniceps*) and Pictorella Finch (*Heteromunia pectoralis*). By contrast, species with low dimension 1 scores, have two lateral palate spots, one medial palate spot, no palate bars, papillae lining the upper and lower gape. Examples of such species include the *Estrilda* waxbills, the *Lagonosticta* firefinches, the *Erythrura* parrotfinches, and the *Pyrenestes* seedcrackers. The mouth marking variables most correlated

with dimension 2 are: the type of swelling on the upper ($R^2 = 0.943$, $p < 0.001$) and lower ($R^2 = 0.942$, $p < 0.001$) gapes, and the number of medial palate spots ($R^2 = 0.218$, $p < 0.001$). Species with high dimension 2 scores have a swollen ridge along the upper and lower gapes and have two medial palate spots. Examples of such species include Red Avadavat (*Amandava amandava*) and Green Avadavat (*A. formosa*).

Ancestral state reconstruction of estrildid mouth markings

Based on the ancestral state reconstructions, the common ancestor of estrildid finches is inferred to have had mouth markings with an ornamentation score of approximately 4.2. This score represents an intermediate level of ornamentation compared to that of extant estrildid finch species. The median ornamentation score of extant estrildid species is 4.33, while the minimum is 0.33 (Orange-winged Pytilia, *Pytilia afra*) and the maximum is 7 (the parrotfinches, *Erythrura* spp.) (Figure 6.8).

The ancestral estrildid finch is inferred to have had: 1 medial palate spot, 2 lateral palate spots, 2 mediolateral palate spots, gape papillae on the upper gape, gape papillae or a generalised swelling on the lower gape and spots on the tongue. It lacked palate swelling, palate bars, connected palate spots and a black ring around the inside of the mouth (Table 6.3)

History of parasitism by Vidua finches

The maximum likelihood reconstruction of parasitism in estrilidid finches shows that there have been at least five independent transitions to being parasitised within the estrildidae (Figure 6.10). These are: 1) in the common ancestor of the genus *Estrilda*, 2) the common ancestor of the genus *Granatina*, 3) the common ancestor of the genera *Lagonosticta, Pytilia, Euschistopiza* and *Clytospiza*, 4) the Zebra Waxbill (*Amandava subflava*), and 5) the African Quailfinch (*Ortygospiza atricollis*). The single apparent loss of parasitism is in the Black-headed Waxbill (*Estrilda atricapilla*). However, while there are no records of parasitism by *Vidua* of this species, it does co-occur with Pin-tailed Whydah (*Vidua macroura*) and the lack of parasitism records is probably an artefact of low observer effort rather than being real (Péron et al. 2016).

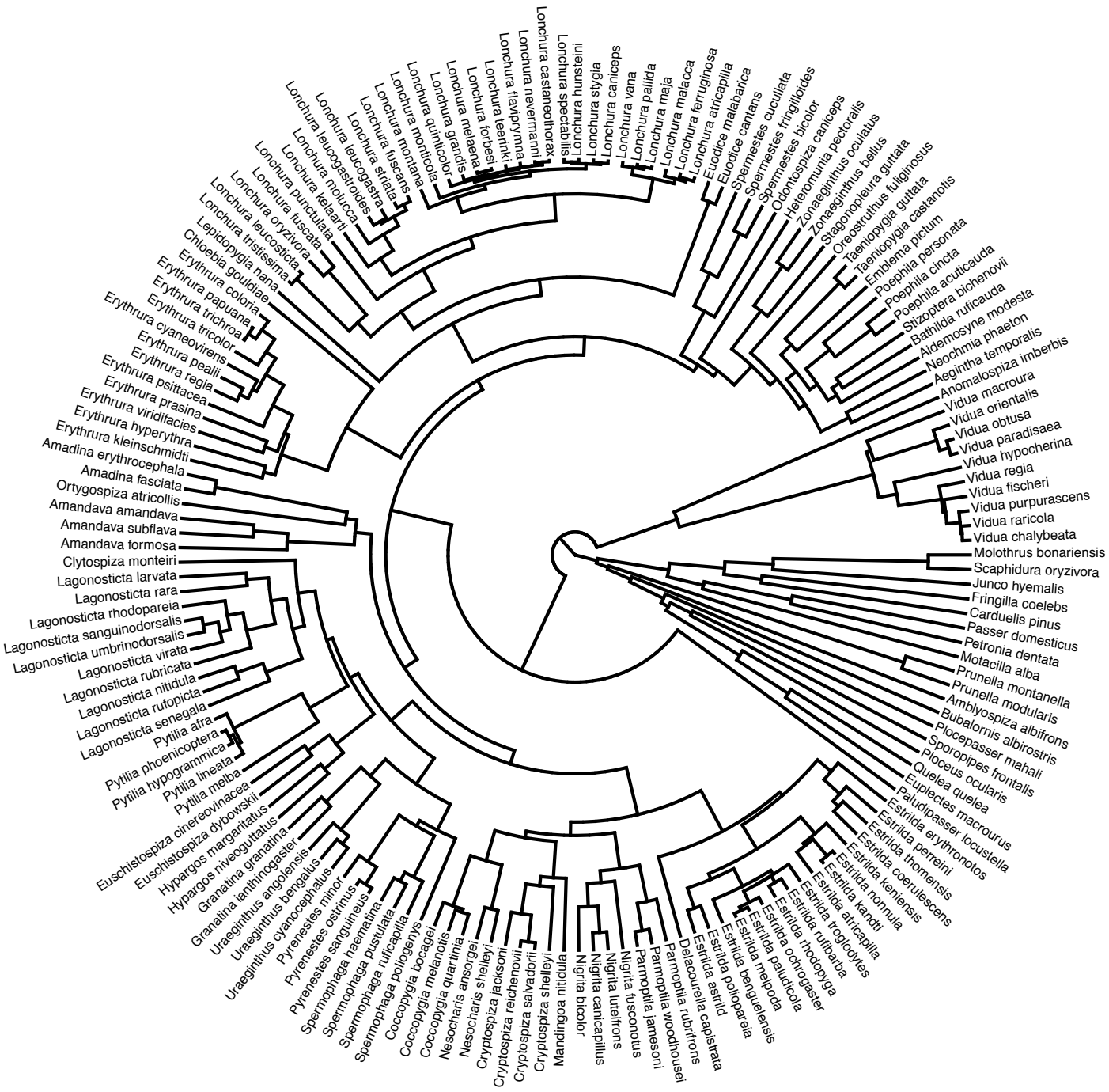

Figure 6.6. mtDNA phylogenetic tree of 141 estrildid finch species and 28 outgroup species produced by Professor Michael Sorenson (see Methods for details on tree construction). Outgroup taxa are *Anomalospiza imberbis* to *Euplectes macrourus*. This is the tree used in this chapter for the comparative analysis. The divergence time between all estrildid finches and the *Vidua* finches is estimated at 15.69 million years ago while the common ancestor of all estrildid finches is estimated at 11.71 million years ago (Gomes et al. 2016).

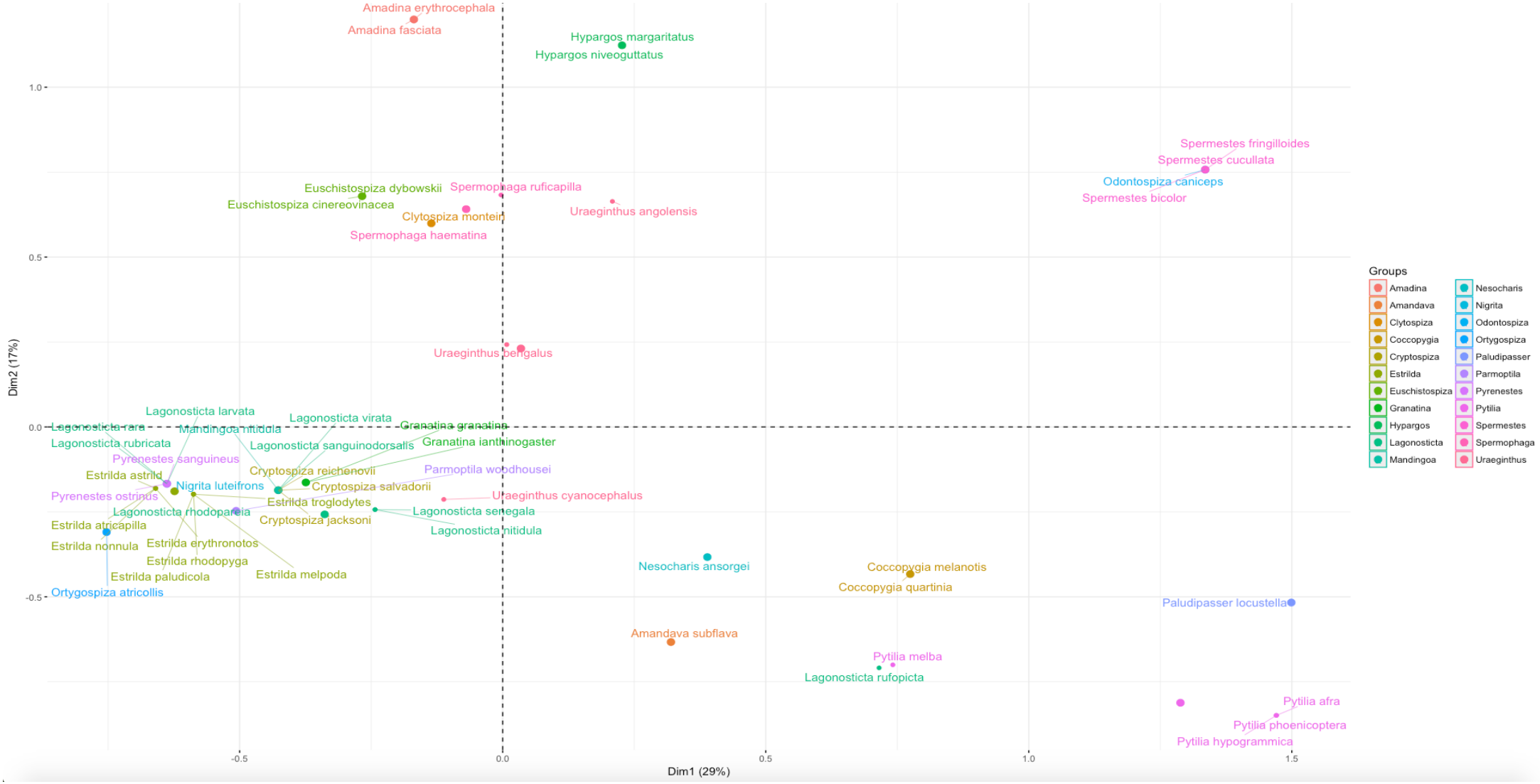

Figure 6.7. Multiple correspondence analysis of African estrildid mouth markings. Based on 8 mouth marking characters: 1) number of medial palate spots; 2) number of lateral palate spots; 3) number of mediolateral palate spots; 4) presence of a palate bar; 5) presence of tongue markings 6) type of swelling on upper gape; 7) type of swelling on lower gape; 8) size of gape swellings. Each point represents a species. The points are coloured by the genus of the species. Dimension 1 is on the x-axis and explains 29% of the total variation in African estrildid mouth markings. Dimension 2 is on the y-axis and explains a further 17% of the variation. Species in the same genus tend to cluster on the plot.

Table 6.3. Inferred ancestral states and measures of phylogenetic signal for each mouth marking trait in estrildid finches. Functions are not available to estimate phylogenetic signal in categorical traits with more than two levels, which is why there is no estimate of phylogenetic signal for four characters. The statistics Bowman's K and Pagel's λ are calculated for continuous traits and ordered categorical traits, whereas the statistic D is used for binary traits. Ordered categorical traits were modelled as continuous to allow K and λ to be calculated.

Character	Ancestral state	K	λ	D
Ornamentation index	4.16	**0.977**	**0.978**	NA
Multiple Correspondence Analysis, Dimension 1	0.188	**2.47**	**0.997**	NA
Number of medial palate spots	1	**2.10**	**1.00**	NA
Number of lateral palate spots	2	NA	NA	**-0.655**
Number of mediolateral palate spots	2	NA	NA	**-0.0676**
Presence of palate bars	Absent	NA	NA	**-0.859**
Size of palate bar(s)	Absent	NA	NA	NA
Type of swelling on upper gape	Papillae	NA	NA	NA
Type of swelling on lower gape	Papillae or general swelling	NA	NA	NA
Size of gape swellings	Small or medium	**1.81**	**1.00**	NA
Presence of tongue markings	Present	NA	NA	**-0.251**
Type of marking on tongue	Spots	NA	NA	NA
Presence of palate swelling	Absent	NA	NA	**-0.913**
Presence of black ring around inside of mouth	Absent	NA	NA	**-0.989**
Palate spots connected	No	NA	NA	**-2.57**

Testing ecological hypotheses on the evolution of estrildid mouth markings (Hypotheses 1, 5, 6 and 7)

All four ecological hypotheses were tested together in a single model using a phylogenetic generalised least squares (PGLS) regression, in which ornamentation index was modelled as a function of the species' parasitism status, clutch size, nesting habitat and whether it nested on the ground or in a tree. The fitted model showed normality and homogeneity of variance in the residuals. None of these variables explained significant amounts of variation in the ornamentation index over and above that explained by phylogeny (see Table 6.4a).

From an information-theoretic perspective, the full model (containing all the ecological co-variates) did not explain the data better than the null model (containing no ecological co-variates) (ΔAIC = +0.87). Additionally, when any of the individual terms were removed from the full model the resultant increase in AIC scores for the model was never greater than 2 (for values see Table 6.4b). This implies that none of the ecological co-variates explained significant amounts of the variation in mouth marking ornamentation.

Table 6.4. Results of phylogenetic least squares regression of host status, nesting habitat, nest location and clutch size on mouth marking ornamentation in estrildid finches. The comparison was carried out on the 93 species of estrildid finch with complete mouth marking information and data available for each of the four ecological co-variates. The results are reported both using significance levels (a) and information theoretic approaches (b). Both show that there is no evidence that any of the ecological factors measured influence mouth marking evolution.

a)

	Estimate	Standard error	t-value	p-value
Host or non-host	0.522	0.495	1.06	> 0.2
Forest or open habitat	-0.440	0.282	-1.56	> 0.1
Ground versus tree nester	0.314	0.204	1.54	> 0.1
Clutch size	-0.280	0.165	-1.70	> 0.05

b)

	AIC	ΔAIC versus null model	ΔAIC versus full model
Null model	274.99	NA	NA
Full model	275.86	+0.87	NA
- Host	275.03	NA	-0.83
- Habitat	276.40	NA	+0.54
- Location	276.35	NA	+0.49
- Clutch size	276.42	NA	+0.56

Effects of parasitism on rate of evolution of mouth marking characters (Hypothesis 2)

The "mouth marking appearance index" (first dimension of the multiple correspondence analysis) was used as the response variable in this analysis. A Brownian Motion model, in which both parasitised and unparasitised lineages have the same rate of mouth marking evolution was found to best explain the data. A model in which rate of evolution was allowed to vary between parasitised and unparasitised lineages fit the data worse than the Brownian motion model ($\Delta AIC = 3.80$, $p > 0.5$). Therefore, there is no evidence that rates of mouth marking evolution vary depending on parasitism status.

Effects of parasitism on speciation rates (Hypothesis 3)

There was no evidence that lineages parasitised by *Vidua* had higher speciation rates than unparasitised lineages. The model in which speciation rates were allowed to vary between parasitised and unparasitised lineages did not fit the data significantly better than one in which speciation rate was constrained to be the same for both states ($\Delta AIC = 1.64$, $p > 0.5$).

Phylogenetic signal in mouth markings (Hypothesis 4)

All mouth marking traits for which phylogenetic signal could be estimated (continuous, ordered categorical and discrete) showed strong phylogenetic signal (Table 6.3). This can also be seen visually when the ancestral state reconstructions are plotted across the estrildid phylogeny (e.g. Figures 6.8 and 6.9).

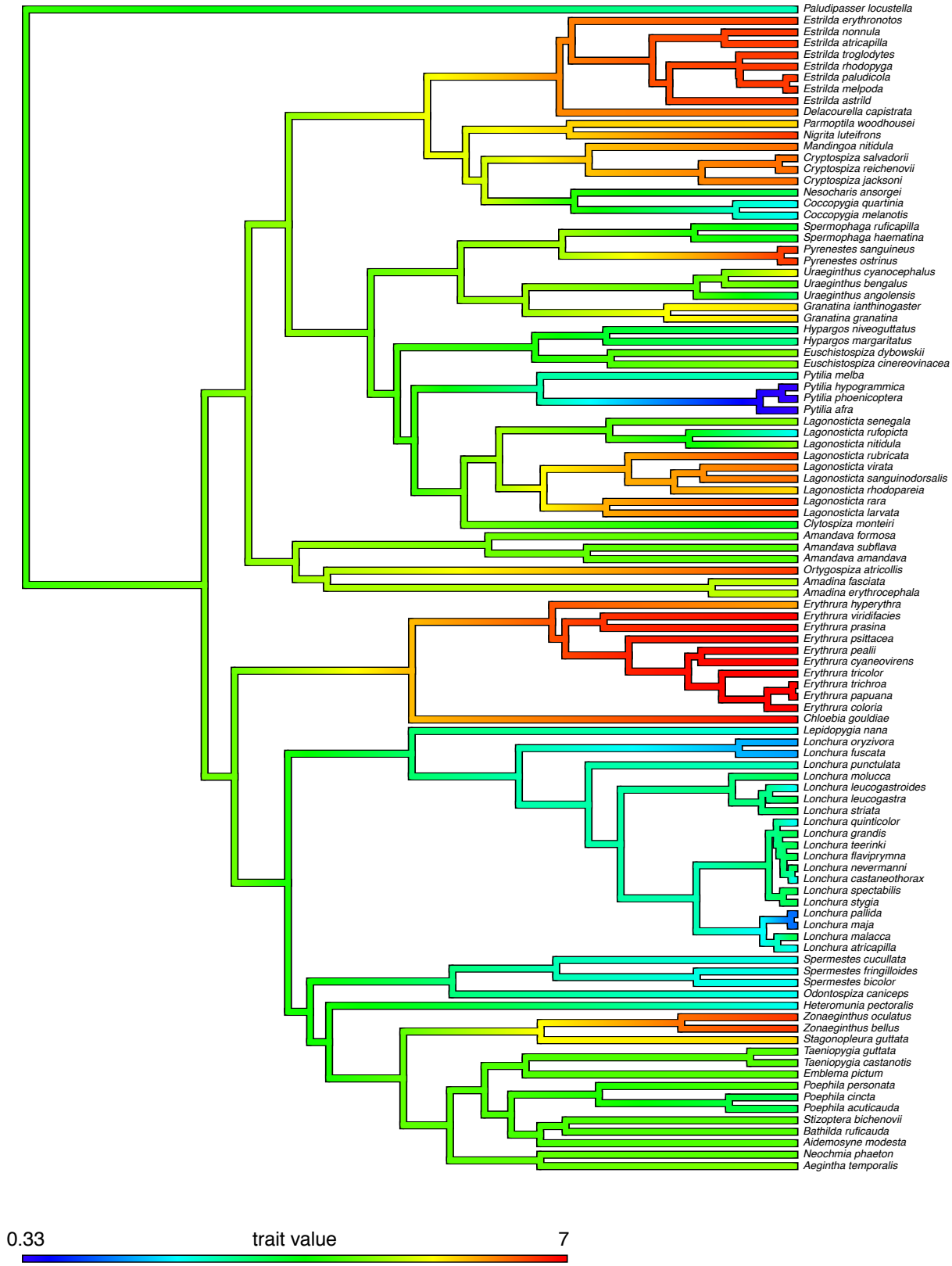

Figure 6.8. Maximum likelihood reconstruction of ornamentation scores across estrildid finch species. The maximum orientation score by an estrildid finch is 7.0 (*Erythrura* parrotfinch species) and the minimum is 0.33 (*Pytilia afra, P. hypogrammica* and *P. phoenicoptera*). The higher the ornamentation score, the more ornamented the mouth marking pattern is for that species. For details on how the ornamentation score is calculated see the methods section in the main text.

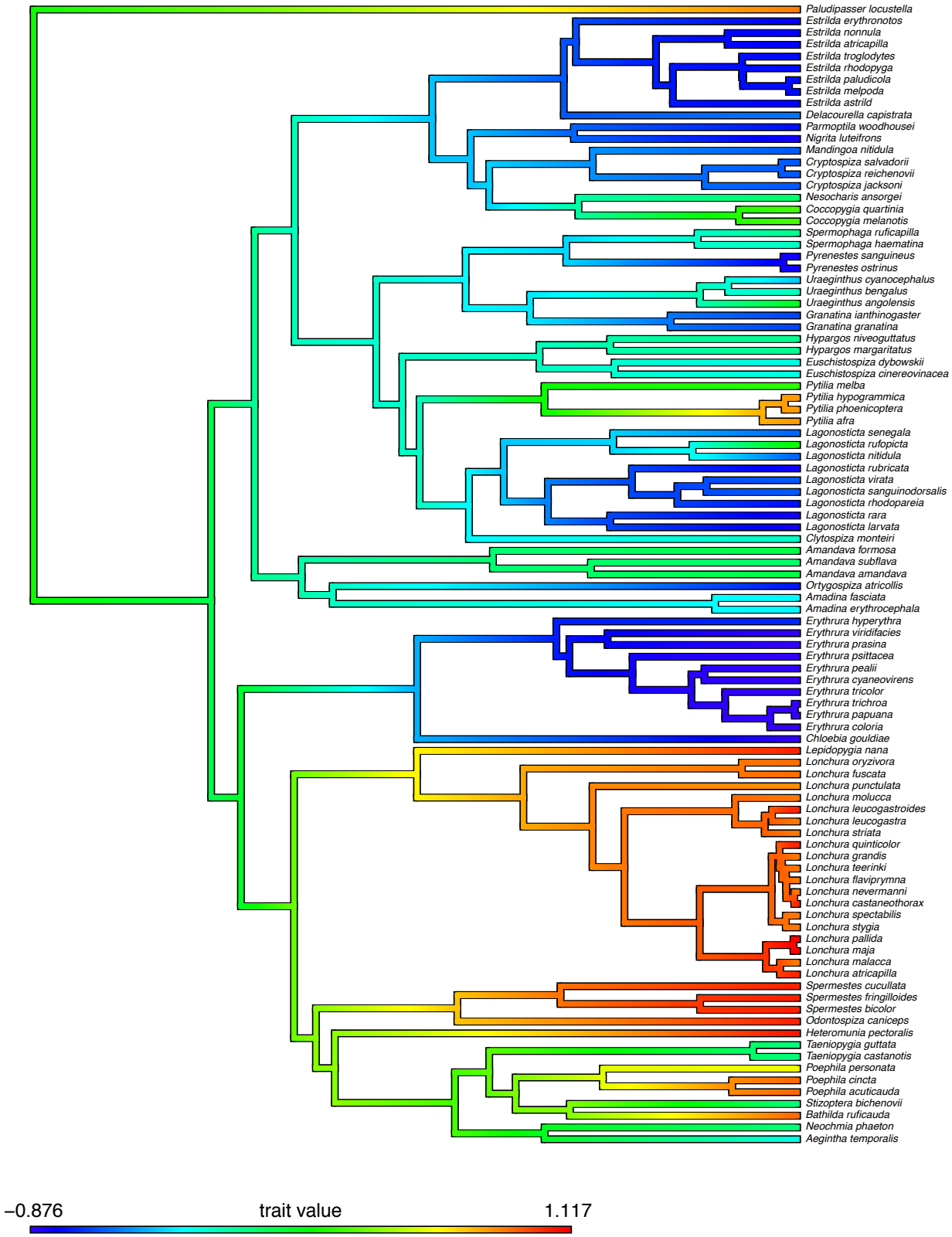

Figure 6.9. Maximum likelihood reconstruction of the mouth marking appearance index (Multiple Correspondence Analysis dimension 1) across the estrildid family tree. Note this is a measure of *type* of mouth marking ornamentation rather than the *degree* of ornamentation (which is plotted on Figure 6.8). Multiple correspondence analysis was done on 8 categorical mouth marking traits (see text for details. Dimension 1 explained 31.7% of the total variation in mouth markings.

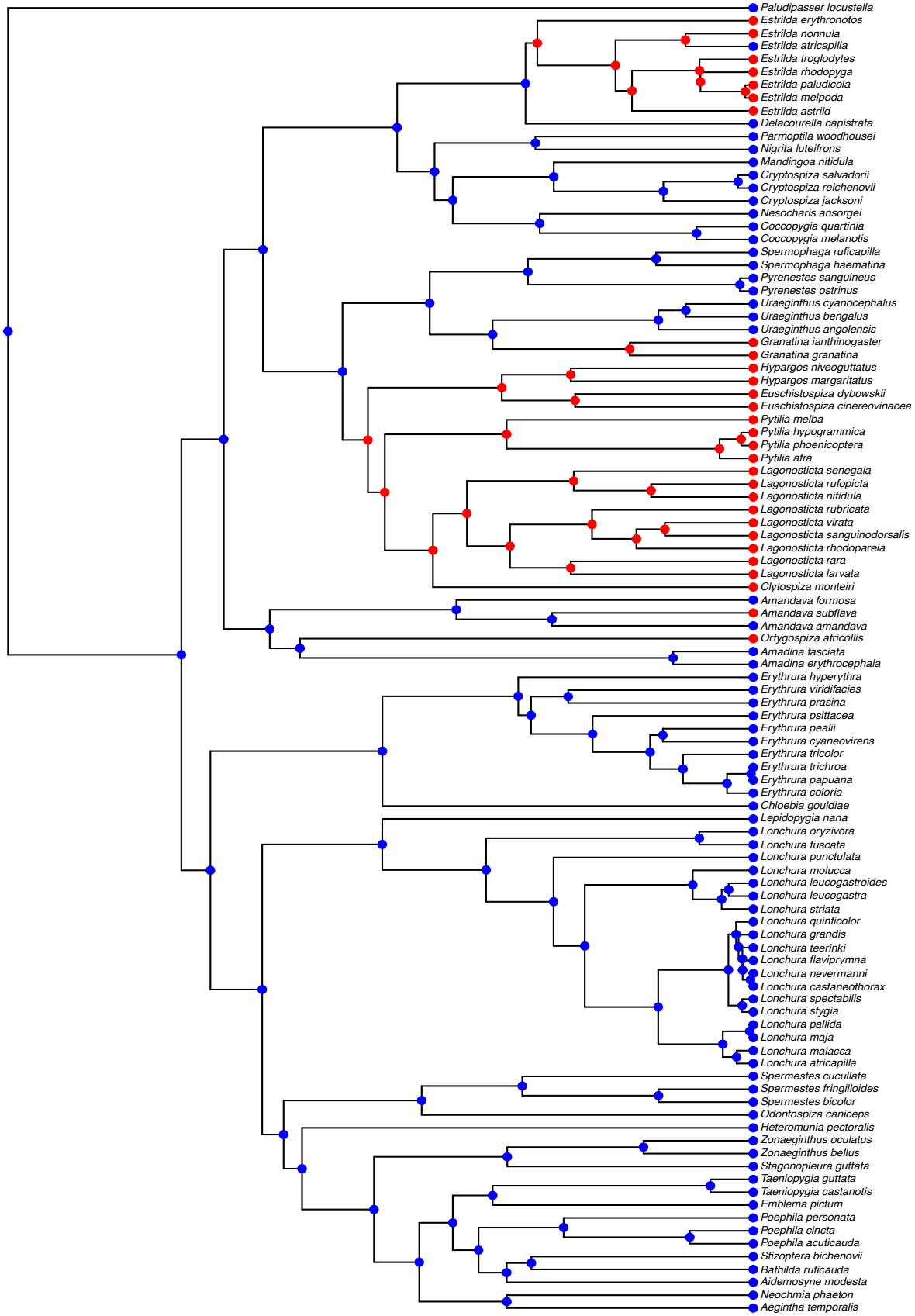

Figure 6.10. Maximum likelihood reconstruction of the history of parasitism of estrildid finches by *Vidua* finches. Red = parasitised; blue = unparasitised.

6.4. DISCUSSION

In this chapter, I carried out a comparative analysis in which I reconstructed the evolution of nestling estrildid finch mouth markings and tested whether several ecological factors have influenced their evolution. The key findings of the chapter are that estrildid mouth markings show very strong phylogenetic signal, that the ecological factors tested/scored have had no detectable influence on estrildid mouth marking evolution and that the ancestral estrildid finch also possessed mouth marking ornamentation.

Phylogenetic clustering in estrildid mouth markings

Phylogenetic signal in mouth markings was strong both when each character within the mouth was reconstructed separately and when composite measures (such as the "ornamentation score" and "mouth marking appearance index") were analysed. This is consistent with Payne's findings of strong phylogenetic signal from his 2005 work on estrildid mouth markings (Payne 2005b) In fact, the phylogenetic clustering observed in the present sutdy was even stronger than would be expected under evolution by Brownian motion across the phylogeny. This was evidenced by K-values greater than 1 for the two continuous mouth marking traits, and D-values of less than 0 for the discrete ones. Phylogenetic clustering in estrildid mouth marking evolution is possibly due to discrimination by estrildid finch parents against odd-looking mouths (Schuetz 2005b, Chapter 5). If estrildid parents discriminate against chicks which do not match an internal template of how their chick should look, then this would lead to strong stabilising selection on chick appearance. This process would result in the pattern of strong phylogenetic clustering of mouth marking traits.

A paradox in the evolution of estrildid mouth markings is therefore as follows: given parental discrimination against novel mouth markings, how does any diversity in mouth markings arise? One way that novelties in estrildid mouth markings can persist may be when environmental conditions reduce the severity of parental discrimination against mismatching chicks. Relaxed parental discrimination could arise when parents breed for the first time and have not yet acquired an internal template of their own chick's appearance. If first-time breeders are less

discriminating, then any environmental conditions that increase the proportion of first-time breeders in the population may allow novel mouth marking phenotypes to enter the population (Langmore et al. 2009; Moskat et al. 2010). Such environmental conditions could include high food abundance in the previous season, resulting in large number of juveniles being recruited to the population. High food abundance in the current breeding season could also lead to relaxed discrimination of mismatching offspring as parents suffer lower costs through misallocating resources to unrelated young than if food is scarce.

That *Vidua* are imperfect mimics of host mouth markings (Chapter 4) suggests that some novelties in chick appearance are not selected against. *Vidua* nestlings can secure sufficient investment from host parents despite having some consistent differences in nestling appearance. These differences may act as a "super-stimulus", even serving to enhance feeding by parents (see Chapter 4 for more discussion on this). Therefore, any novelties in the estrildid populations that act as "super-stimuli" could increase the fitness of the chick.

The lack of an effect of ecological factors on estrildid mouth marking evolution

There are several possible reasons for the absence of a detectable influence of ecological factors measured influenced the evolution of mouth marking ornamentation.

First, the variables used to measure sibling conflict, predation risk and light environment (clutch size, nest height and nest habitat respectively) were admittedly distant proxies of the key ecological factors being investigated. Were finer measures of each of these available, it is possible that a relationship between them and mouth marking evolution could have been detected. However, obtaining these finer measures for most estrildid species would not be logistically feasible as it would require many years of detailed, long-term fieldwork across multiple continents. It might be possible to obtain some of this information for a subset of the species.

Second, transitions between states in the ecological characters may have occurred too rarely in the estrildid family tree to give us enough power to detect their effects on mouth marking evolution. Based on the ancestral state reconstructions,

there were only five independent transitions to parasitism in the estrildid family tree. The two most ancient ones were at the base of the *Estrilda* genus (comprising seven currently parasitised species) and at the base of the *Lagonosticta*, *Clytospiza, Euschistopiza, Pytilia* and *Hypargos* genera (comprising 18 currently parasitised species). The remaining three transitions account for just four more species. Similarly, there have only been 5 ancestral transitions between ground and tree-nesting in estrildids and 3 ancestral transitions between open grassland and forest habitats. This reduces the statistical power to detect the impacts of each of these ecological switches on mouth marking evolution.

Third, the way in which nestling mouth markings were scored was imprecise. The current analysis focussed only on mouth marking pattern and not on colour. The fact that *Vidua* mimic both colour and pattern of the specialist hosts suggests that both are necessary to solicit adequate feeding from parents. Birds perceive colour differently to humans and so attempts to quantify colour diversity by humans using subjective and categorical assessments are likely to be inaccurate. The UV and non-UV photographs analysed in Chapter 4 provide a potential way forward to quantify colours in multiple estrildid species. If the number of species for which these standardised photographs are available increases, it would be possible to carry out a comparative analysis that also quantifies colour diversity.

Finally, estrildid nestling mouth marking evolution may truly not be influenced by ecology. The possibility of non-ecological divergence in traits associated with social interactions (such as male ornamentation traits, weapons and flowers) has been explored by West-Eberhard (1983) and Prum (2010). Patterns of diversification of nestling mouth markings may instead represent arbitrary shifts in parental preferences. Lyon et al. (1994) hypothesised that American Coot (*Fulica americana*) may prefer ornamented chicks not because their ornamentation conveys any honest information about chick quality or condition but instead because the colour exploits pre-existing sensory biases of parents. Elaboration may then follow through a positive feedback cycle between nestling trait and parental preference in a process analogous to Fisherian runaway selection (Drown and Wade 2014; West-Eberhard 1983). However, in the context of estrildid finches, it is not clear whether

any parental sensory biases exist and whether these are arbitrary or grounded in the species' ecology.

That nestling mouth markings are primarily exposed to parents within the enclosed environment of an estrildid nest could also explain the lack of an effect of nest habitat. Estrildid nests are generally quite similar across species, most possessing a domed structure. This would mean that, if a bird lived in interior forest or an open savannah much of this variability would not translate into within nest light environment. This could be tested by measuring the light environment within estrildid nests using a spectrophotometer. However, estrildid finches do retain their mouth markings post fledging when they are still fed by parents as they move through their natural habitat. In this context, ambient light environment could still play an important role.

Whether these shifts result from mutations or different learning experiences on the part of the parents will depend on how parental preferences for nestling appearance are acquired. If parental preference is innate, then offspring will likely inherit their parents' preference. Additionally, given that the offspring were able to survive to fledging in their parent's nest, it is likely that they too possess the genes for nestling appearance that their parents prefer. This means that the offspring surviving best in a nest will both have the genes for the trait and for the preference of that trait. This would be especially the case if the genes influencing trait and preference were close to one another on a chromosome such that the chance of recombination breaking them up was lowered.

The connection between parental preference and offspring trait could be somewhat diluted because the finch may mate with another finch that has slight differences in preference and confers slightly different genes for nestling appearance to offspring. However, if this happened then parents could discriminate among offspring and allocate most food to chicks matching their ideal internal template of chick appearance. This should, on average, select against non-conforming chicks and produce fledglings that have both trait and preference. If mutations in parental preference occur, this could select for changes in nestling appearance over generations. It is likely that mutations in preference would lead the co-evolutionary

walk of trait and preference, because changes in appearance without changes in parental preference are selected against. The model is different if parental preference is determined instead by parental experience with their first broods. Under this situation, parental preference is likely to be a lot more labile through plasticity, but also less heritable. It would be interesting to construct models of the evolution of nestling appearance under conditions where parental preference is assumed to be innate and where it is assumed to be plastic. The predictions of the model could be tested against the pattern of diversity seen in estrildid finch nestlings.

The ancestral estrildid showed mouth marking ornamentation

Reconstruction of the ancestral state for each mouth marking characteristic revealed that the ancestral estrildid finch had intermediate levels of ornamentation compared to the extant species and that there have been both gains and losses of ornamentation levels over the estrildid phylogeny.

Future work to re-construct the mouth markings of the ancestral *Vidua* finch would be very useful in understanding the radiation. Which modern day group of estrildid finches did the mouth markings of the ancestral *Vidua* most closely resemble? This could help us understand where process of host colonisation by *Vidua* began. It could also help us to understand why the Cuckoo Finch (*Anomalospiza imberbis*) has no mouth marking ornamentation. Did this represent a loss of ornamentation in the lineage leading to this species or did the common ancestor of *Vidua* and *Anomalospiza* lack ornamentation? The hosts of Cuckoo Finches, prinias and cisticolas, have young that lack any conspicuous ornamentation besides spots on the tongue. It is possible that a loss of ornamentation in Cuckoo Finches represented adaptation to these new hosts. Currently, attempts to reconstruct the evolution of mouth markings in *Vidua* finches are confounded by the *Vidua* phylogeny, especially amongst the indigobirds being poorly resolved (DaCosta and Sorenson 2016). This is due to the ongoing introgression between *Vidua* species and the fact that some *Vidua* species are young (Balakrishnan et al. 2009). Additionally, there may have been many lineages of *Vidua* which colonised and adapted to other estrildid finch species which have subsequently gone extinct. It may be that the dynamics of host colonisation and extinction in *Vidua* results in many of its species having a more fluid and transient nature than is found in most other adaptive radiations.

Chapter 7:
Conclusions

Chapter 7: Conclusions

In this thesis, my aim has been to investigate the processes that limit adaptive radiations. I focussed particulary within the *Vidua* finches, a radiation of brood-parasitic passerines endemic to Africa. Chapters 2 and 3 explored broad conceptual themes concerning the nature and development of mimicry. Mimetic resemblances are often key adaptations to facilitate the successful colonisation and long-term exploitation of new host environments by parasites. Chapters 4, 5 and 6 focussed specifically on the *Vidua* radiation, using a mixture of field experiments, comparative analysis, UV photography, sound recording and DNA barcoding to shed light on this system. I will begin by reviewing the key findings from each chapter, and then go on to explore the broader implications of these results for the main questions posed in this thesis.

A review of the key findings of this thesis

Following from the Introduction, I began in Chapter 2 by critically examining the logic with which mimicry can be conceptually organised and analysed. Mimicry underpins the host-specific adaptations that *Vidua* finches have evolved to successfully survive in novel host environments. I highlighted three evolutionarily relevant distinctions: 1) Are the model's traits being mimicked signals or cues? 2) Does the mimic signal a fitness benefit or fitness cost in order to manipulate the receiver's behaviour? 3) Is the mimic's signal deceptive? The first distinction divides mimicry into two broad categories: "signal mimicry" and "cue mimicry". "Signal mimicry" occurs when mimic and model share the same receiver, and "cue mimicry" when mimic and model have different receivers or when there is no receiver for the model's trait. The second and third distinctions divide both signal and cue mimicry into four types each. These are the three traditional mimicry categories (aggressive, Batesian and Müllerian) and a fourth, often overlooked, category for which the term "rewarding mimicry" is suggested. Rewarding mimicry occurs when the mimic's signal is non-deceptive (as in Müllerian mimicry) but where the mimic signals a fitness benefit to the receiver (as in aggressive mimicry). The existence of rewarding mimicry was found to be a logical extension of the criteria used to differentiate the

three well-recognised forms of mimicry. These four forms of mimicry are not discrete, immutable types, but rather help to define important axes along which mimicry can vary.

In Chapter 3, I focussed on a specific adaptation relevant to avian brood parasites and their hosts – begging call mimicry. This provided clear predictions about whether we expect mimicry of host begging calls by *Vidua*, and whether we expect that mimicry to develop primarily through environmental or genetic inputs. I began by reviewing the literature on reported similarity between the begging calls of avian brood parasites and their hosts. This survey highlighted how many species of avian brood parasite still have not had their begging calls described (63%). I showed that such similarity is a more widespread phenomenon than previously appreciated. Secondly, I examined the selection pressures that drive the evolution of begging call mimicry by avian brood parasites, assess their importance, and illustrate them with empirical examples. Finally, I proposed a theoretical framework to explain variation in the ways that brood parasite begging calls develop. The framework suggests that the mode of development can be predicted from a consideration of the accuracy of genetic cues (as mediated by parasite specialisation levels) and the benefits to the young parasite of using environmental cues to modulate their begging call (as influenced by levels of discrimination shown by host parents). The specialist nature of most *Vidua* species, coupled with the discriminatory behaviour of host parents against mismatching chicks, means we expect *Vidua* to have mimetic mouth markings that develop primarily through genetic cues. This lack of plasticity in begging calls would likely make colonisation of host species with very different begging displays even more difficult for *Vidua*, because the parasite is unable to compensate for its deficient visual signals by rapidly developing mimetic calls.

In Chapter 4, I explored the mimicry of host nestlings by *Vidua* finches in three different traits belong to two sensory modalities. In addition to providing further/quantitative evidence on visual mimicry, I provided the first quantitative evidence that at least three species of *Vidua* finch (Pin-tailed Whydah, Broad-tailed Paradise Whydah and Purple Indigobird) mimic the begging calls of their respective hosts. I also provided qualitative evidence of postural mimicry of head movements given during begging display. To better understand the selection pressures

experienced by *Vidua* from host parents, I examined whether there are consistent differences in the mouth markings and begging calls of one *Vidua*-host pairing; namely, between Pin-tailed Whydah and its host, Common Waxbill. I found evidence that Pin-tailed Whydah are in fact imperfect mimics of their hosts, since there are consistent differences in both the mouth marking patterns and the begging calls between parasite and host. I explored potential evolutionary explanations for this discrepancy and outlined experiments that could help to distinguish among these different hypotheses. That all three species of *Vidua* investigated showed evidence of host mimicry in mouth markings, begging calls and possibly head movements, suggests that, to successful colonise a new host and obtain sufficient investment from parents, *Vidua* must ultimately evolve mimicry in all three traits. This increases the barrier for successful colonisation of a new host compared to if mimicry in just a single trait was sufficient (Gilman et al. 2012).

In Chapter 5, I simulated the colonisation of a new host by experimentally transferring Pin-tailed Whydahs into the nests of Blue Waxbills (a rarely parasitised estrildid finch species). I found that Pin-tailed Whydahs survived poorly in the new nest environment, and much worse than Blue Waxbill chicks transferred to other Blue Waxbill nests. There was no difference in survival in the Blue Waxbill nest environment for transferred Pin-tailed Whydah and transferred Common Waxbill chicks. Common Waxbills are the natural hosts of Pin-tailed Whydah and possess similar begging displays (see Chapter 4). The lack of a difference here suggests that Pin-tailed Whydahs do not have any additional adaptations to solicit more food from parents or be more robust in a new nest environment compared to an estrildid finch species with similar begging displays. The discrepancy in survival between Pin-tailed Whydahs and Blue Waxbills in Blue Waxbill nest environments seemed to be due to Pin-tailed Whydah nestlings being fed less food by parents than were Blue Waxbill nestlings, rather than because there are important dietary differences between that provided by Common Waxbill parents (the natural host environment) and that by Blue Waxbill parents. There was no evidence that Pin-tailed Whydahs developed different call types having been raised in a Blue Waxbill nest compared to being raised in a Common Waxbill nest. This suggests that the mimicry of Common Waxbill nestling begging calls by Pin-tailed Whydahs is innate and therefore that large-scale plasticity is not available to help with host switches and so facilitate

speciation. However, certain call types within the Pin-tailed Whydah nestlings natural repertoire were used more frequently in Blue Waxbill nests than in Common Waxbill nests. Particularly, a rapid high-pitched call type was employed by Pin-tailed Whydahs in Blue Waxbill nests over a much wider period of development than when raised in Common Waxbill nests. This could be an example of Pin-tailed Whydahs choosing the call types from within their natural repertoire that best elicit feeding from the new host parent. Additionally, there were some slight shifts for certain call parameters within some of these call types depending on the host rearing environment. These differences could not be explained merely by the Pin-tailed Whydahs in the Blue Waxbill nest being hungrier than those in the Common Waxbill nest, host environment. Whilst these slight shifts may have been attempts by the whydah nestling to solicit greater investment from a new host parent they did not result in increased survival compared to Common Waxbill nestlings in the new host environment. This provides additional evidence that host switches, especially between hosts with very different begging displays, are hard and that parasites may have to rely on a loophole of relaxed parental discrimination when food is abundant or parents are naïve.

Finally, in Chapter 6, I reconstructed the evolution of nestling mouth markings in the estrildid finches (the family to which the hosts of *Vidua* belong). This revealed strong phylogenetic signal in mouth markings across the estrildid phylogeny, which may explain why successful colonisations of new hosts by *Vidua* tend to be of species that are in the same genus (or at least the same clade) as the ancestral host ("clade-limited colonisation") (Sorenson et al. 2004). This is consistent with my experimental findings in Chapter 5, that a *Vidua* species adapted to exploit a member of the genus *Estrilda* was unable to successfully colonise a member of the genus *Uraeginthus.* None of the contextual variables scored (parasitism by *Vidua*, sibling competition, light environment and predation rate) had a detectable effect on mouth marking evolution in estrildid finches. This suggests either that the proxies used for these ecological variables and mouth markings were not accurate enough, or that mouth marking evolution in estrildid finches really does evolve independently of these aspects of their ecology. Therefore, the amazing diversity of estrildid mouth markings remains somewhat of a mystery. There is no evidence that the complex mouth markings (which the results of Chapter 5 suggest now act to foil parasitism by *Vidua*)

evolved for that purpose or have been subsequently modified over evolutionary time in response to parasitism. Future work to better understand this diversity will have to focus on more accurately quantifying the ecology of estrildid finches (e.g. using a spectrophotometer to quantify light environments within nests) and obtaining quantitative ultra-violet photographs for a greater variety of estrildid species. Additionally, the predictions of non-ecological models of the nestling diversification in response to interactions with parents must be investigated to see whether these can explain this remarkable radiation of offspring characters.

An integrated picture of host colonisation and speciation in *Vidua*

Taken together, these findings present the following picture of the *Vidua* radiation. As nestlings, many *Vidua* mimic the mouth markings, begging calls and, possibly, head movements of their hosts (Chapter 4). As predicted by the framework outlined in Chapter 2, these mimetic traits develop primarily through genetic cues with only slight changes depending on host environment (Chapter 5). These mimetic adaptations can be classified as "aggressive signal mimicry" according to the framework presented in Chapter 2. It is signal mimicry because mimic and model share the same intended receiver (the host parent) and the mimic is therefore copying a *signal* of the model. It is "aggressive" mimicry because the *Vidua* nestling is deceptively signalling the promise of a reward to host parents. Host parents are manipulated into feeding *Vidua* finches under the illusion that this investment will increase their own lifetime reproductive success. However, in reality, this investment is wasted and actually harms the parents' reproductive success by depriving their true offspring of food.

A key barrier to host colonisation, and therefore speciation, for *Vidua* seems to be persuading host parents to feed them adequate *amounts* of food rather than the right *kind* of food (Chapter 5). The strong phylogenetic signal in nestling mouth markings (and presumably other begging display traits) potentially explains why successful colonisations tend to be to species closely related to the ancestral host (Payne 2005b; Sorenson et al. 2004) . Certain clades that are seemingly suitable for colonisation have presumably not been colonised because their mouth markings are too dissimilar and the initial fitness barrier to entry is too high. For example, no

genera with a bar rather than spots on the upper palate (such as the genera *Spermestes, Paludipasser* and *Euodice*) are currently known to be parasitised. Additionally, habitat seems to provide an important filter limiting which estrildid finches become parasitised by *Vidua* finches, so unlocking speciation. For example, none of the forest-living African estrildids (such as those in the genera *Cryptospiza, Mandingoa, Nesocharis, Nigrita, Parmoptila* and *Spermophaga*) are regularly parasitised. Two African estrildids living in dense thicket habitat, Red-throated Twinsot (*Hypargos niveoguttatus*) and Pink-throated Twinspot (*H. margaritatus*) are regularly parasitised by indigobirds. Thicket habitat is often bordered by more open savannah habitat, so it may be that this allowed *Vidua* to colonise these species, even though *Vidua* have not being able to exploit species inhabiting the interior of forests. This does suggest, however, that as pristine forest is broken up and exposed to more open habitat through the actions of people cutting down trees, it could allow *Vidua* colonise to previously inaccessible host species (Péron et al. 2016). This could occur in a manner similar to Brown-headed Cowbirds (*Molothrus ater*), for whom forest fragmentation has allowed increased parasitism rates of existing hosts as well as increased access to new undefended ones (Robinson et al. 1995).

The absence of *Vidua* parasitising members of the genus *Uraeginthus* (which includes the Blue Waxbill used in the transfer experiments of Chapter 5) is more puzzling. *Uraeginthus* waxbills are common in the same habitat as many *Vidua* species, but are only rarely parasitised. The results from Chapter 5 suggest that the mouth marking possessed by Pin-tailed Whydahs (which mimic those of Common Waxbill) are too dissimilar to those of Blue Waxbills for Pin-tailed Whydahs to be fed adequate amounts of food. Blue Waxbill mouths differ from Common Waxbill mouths in that Blue Waxbills have just three (rather than five) spots on the upper palate and a blue (rather than pink) background colour. In addition, Blue Waxbills lack the conspicuous white arcs on the upper gape and white papillae on the lower gape of Common Waxbills. Instead they have just a small dark swelling (see Figure 5.1 for photos of the mouth markings of Blue Waxbill and Pin-tailed Whydah and Figures 4.6 and 6.1 for comparison of Blue Waxbill and Common Waxbill mouths). Having three upper palate spots should not be a challenge for *Vidua* to evolve, since village Indigobirds that mimic Red-billed Firefinches have evolved three upper palate spots in a pattern similar to that of Blue Waxbill (Payne 2005b). However, no *Vidua*

has evolved to match the blue upper palate colour of Blue Waxbills (and other *Uraeginthus* species). It is possible that this represents a barrier to successfully exploiting these species. To test whether the blue background palate colour is important for survival in a Blue Waxbill nest, an informative experiment would be manipulate this trait in Blue Waxbill nestling mouths and see how this influences their survival. If Blue Waxbills with even slightly manipulated mouths don't survive well compared to sham-manipulated controls, it would suggest that Blue Waxbill parents are highly discriminating against mismatching offspring.

Whilst there is evidence that many estrildid finch parents do discriminate against odd-looking offspring, there have, as yet, been no quantitative comparisons of which estrildids are the most discriminating or even whether estrildid species vary in their degree of discrimination. For example, are estrildids subject to parasitism pressure from *Vidua* more discriminating than those which are not? An ideal experiment to test this would be to compare the levels of discrimination shown by Common Waxbills that co-occur with their parasite, the Pin-tailed Whydah, and those which do not. Common Waxbills have been shipped by humans to many islands in the Pacific Ocean. Pin-tailed Whydahs have also been translocated to some of these, such as Réunion. On other islands, the waxbill occurs alone (Fry and Keith 2004). It would be fascinating to compare the levels of discrimination (as well as the patterns of variation in mouth marking and begging calls) shown by Common Waxbills in these different environments. We might predict that discrimination against odd-looking chicks would be costly in an environment lacking parasites and so might be lost when parasitism pressure is eased. The methodological approach would be a similar approach to the one employed by David Lahti who compared egg rejection behaviour and egg phenotypes of Village Weavers (*Ploceus cucullatus*) between mainland Africa (where they are subject to parasitism by Diderick Cuckoos (*Chrysococcyx caprius*) and islands in the Caribbean where they occur without their brood parasite (Lahti 2005; Lahti 2006).

The role of hybridisation between *Vidua* lineages in the origins of novel *Vidua* mouth marking features has yet to been investigated. The remarkable imprinting system possessed by *Vidua* sets the stage for speciation if new hosts are colonised, but also for hybridisation if hosts used by other lineages are accidentally used. Hybrid

Vidua are known even from species that are not closely related within the *Vidua* radiation. For example, Payne (1980) observed hybrids between Purple Indigobirds and Long-tailed Paradise Whydahs. That these species from different ends of the *Vidua* radiation can produce apparently viable offspring suggests that there are no intrinsic mating barriers between *Vidua* species. Therefore, if one *Vidua* species evolves a novel mouth marking innovation through mutation, it has the potential to be spread and recombined with the mouth markings of other *Vidua* lineages to form new and potentially adaptive mouth markings. The importance of hybridisation in the origins of adaptive innovations and ecological transitions is now widely appreciated (Mallet 2007; Rieseberg et al. 2003; Seehausen 2004). As genomic tools improve and become cheaper, we will be able to study the genomes of *Vidua* in finer detail and better assess the extent of ongoing hybridisation between lineages. Crosses between *Vidua* could also be carried out in captivity and the mouth markings of offspring investigated. Not only would this help shed light on the genetic architecture of various mouth marking traits, but these hybrid chicks could then be introduced to different host parents to test the plausibility of this mechanism for facilitating host colonisation and speciation. *Vidua* finches are probably the only brood parasite system for which classical genetics is possible to investigate the genetic basis of mimetic traits. The hosts and the parasites are both seed eaters, mating preferences can be manipulated by changing the host rearing environment, and the species have been kept successfully in captivity in the past (Nicolai 1964; Payne et al. 1998; Payne et al. 2000; Payne et al. 2001). Additionally, specialist lineages of *Vidua* adapted to the same host species are not necessarily monophyletic (DaCosta and Sorenson 2016). For example, Village Indigobirds in west and southern African both parasitise and mimic the appearance of Red-billed Firefinch (Payne 1985). However, the two populations of *Vidua* are not monophyletic (DaCosta and Sorenson 2016). This suggests that either that each lineage has independently acquired the necessary mimetic adaptations to exploit Red-billed Firefinches or that there has been some introgression of characters between lineages in the past.

Why are there only 19 species of Vidua?

Returning to the major question posed at the start of this thesis: why have some radiations diversified more than others? In the context of *Vidua* it seems a

combination of habitat filters, the complexity of host begging displays, the strong phylogenetic conservatism of host begging displays, the discrimination by estrildid parents against odd chicks and the lack of adaptive plasticity in begging display traits by *Vidua* have combined to limit the radiation to consist of just 19 species rather than having diversified to a far greater extent. *Vidua* finches are a useful system to address this question in because we have a clear understanding of the different ecological niches ("host species") they can inhabit and the varying selection pressures exerted by each environment. Additionally, the imprinting mechanism displayed by *Vidua*, provides a clear link between niche colonisation and reproductive isolation. This connection is not always as straightforward in other radiations where reproductive isolation instead evolves primarily as a by-product of local genetic adaptation to the novel environments over generations (reviewed in Nosil 2012).

This work in this thesis emphasises the importance of detailed natural history studies combined with field experiments, comparative analysis and theoretical work to understand the processes underpinning adaptive radiations. The knowledge generated from such research is what allows the landscape of potential niches and their respective selection environments to be mapped, whilst also providing insights into the link between colonisation and the origin of new species.

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