The evolution of acoustic identity signals in birds

Dissertation

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Summary

The focus of this dissertation is on the evolution of phenotypic variation resulting not from natural or sexual selection, but from selection for 'distinctiveness'. Historically, the main paradigms of evolutionary biology rooted in natural and sexual selection trying to explain a large proportion of phenotypic variation occurring between conspecifics; however, there are individual differences that are left unexplained by these selective forces. Some of these phenotypic differences allow individuals to recognize each other by making its bearer distinguishable from others. Distinctiveness is a selective advantage whenever two individuals benefit from repeated interactions, but face the risk of mistaking one individual for another.

Individual recognition has been demonstrated across many taxa and in different sensory modalities. Probably the best known and most impressive example, illustrating the benefits of individual recognition, are breeding colonies of birds. In a penguin colony of thousands, without landmarks or nests and over constant background noise from hundreds of conspecific young, a chick is able to recognize its parents by call even after weeks of separation. The ability of chicks and parents to recognize each other using complex contact calls is a fascinating communication system to study the evolution of identity signals.

In this dissertation, I set out to answer questions about different aspects of identity signalling from a signaller's and a receiver's perspective in different songbird study species. The first two chapters examine from a signaller's perspective how acoustic parameters can be influenced by a chick's condition as well as by its genetic background and rearing environment. First, I present findings on the influence of hunger on the acoustic individuality in begging calls of nestlings of colonially breeding weaver birds (Chapter 1). Our results demonstrate for the first time how acoustic individuality is preserved in calls while simultaneously indicating a chick's hunger to its parents. These findings further suggest that familiarity with a chick's begging calls has the potential to increase the parents' assessment of a chick's hunger state.

After finding that condition (i.e. hunger) can influence certain acoustic parameters while others remain stable, we investigated the influence of genetic background and environmental influences on these parameters in a cross-foster study on wild tree swallows (Chapter 2). Siblings within a brood often share a common acoustic features referred to as brood signatures. In our study we found that nestlings that were raised by their biological parents showed a brood signature, while calls of cross-fostered nestlings did not, neither for their nest of rearing nor for their nest of origin. On the level of acoustic parameters, our study revealed that variation in specific call parameters can be partly explained by effects due to a common origin (e.g. genetic effects) while others by the rearing environment (e.g. social interactions) of the nestlings.

The studies of the next three chapters adopt a receiver's perspective and investigate the accuracy of individual recognition, and why and when individuals commit recognition errors. In a laboratory study on zebra finches, I demonstrate that fledglings are able to identify their parents by contact calls (Chapter 3). However, fledglings do not exclusively respond to their parents, but respond also to unrelated adults, suggesting that fledglings make recognition errors, possibly related to the acoustic similarity of adult individuals. Surprisingly, in playback experiments with calls of own and unrelated young, zebra finch parents, did not seem to respond primarily to contact calls of their own fledglings (Chapter 4). This suggests that parent-offspring recognition is either a one-sided process or that parents avoid vocal contact under situations such as simulated by our playback setup.

Following the finding from chapter 3 on a potential relationship between acoustic similarity and the likelihood for recognition errors, we tested this hypothesis in a large scale playback experiment with zebra finches (Chapter 5). By presenting a series of adult calls of know similarity to fledglings, we were able to demonstrate that acoustic similarity is related to 'false responses'. This finding demonstrates for the first time that acoustic similarity in contact calls of birds can cause recognition errors. Collectively, our results provide support for the hypothesis that evolution for distinctness is driven by negative-frequency dependent selection.

In summary, in this dissertation I present several novel findings on the evolution of acoustic identity traits in birds, with a special focus on parent-offspring communication. I present novel insights on how vocalizations are influenced by condition, environment and genes, and which acoustic parameters are used to form unique identity signatures. Furthermore, I provide first experimental evidence for the role of acoustic similarity for recognition errors in birds, supporting the importance of negative-frequency dependent selection for the evolution and spread of individual signatures.

General introduction

Communication is often viewed as an evolutionary game played between a signaller and a receiver. In its basic form an individual signals information, typically about its own identity, quality or condition, and the signal receiver interacts with the signaller, with a payoff that depends on this information (Maynard-Smith and Harper 2003). Signals may vary in many different ways ranging from the composition of chemical compounds in insect pheromones to elaborate territorial songs in many oscine birds. The functional significance of inter- and intraspecific variation in signals has already been the focus of discussion and debate in the late nineteenth century (Darwin 1871; Blaisdell 1992). The vast majority of published studies have tried to understand the function of signals in relation to sexual selection processes.

While sexual selection certainly is an important selective force in shaping signals, research has largely overlooked an important alternative that explains population variance in phenotypic traits: the selection for individual recognition and herewith identity traits (Johnstone 1997; Dale et al. 2001). Individual recognition occurs whenever an organism identifies another individual according to its distinctive characteristics. From this conceptual point of view, individual recognition is not exclusively limited to one specific individual but is extensible to recognition of individuals belonging to a certain group that share a common signal. The level of recognition can therefore reach from species recognition where all individuals of a species share a common signal (species identity) over sex, kin, neighbour, rival, down to individual recognition (individual identity) when every individual has unique cues and is thereby reliably identifiable (Dale et al. 2001). The ability to identify particular individuals is a widespread phenomenon that has been shown in a number of species. Both observational and experimental evidence exists for birds (Watt 1986; Whitfield 1986; Dhondt and Lambrechts 1992; Collias 1993), fish (Hojesjo et al. 1998), mammals (Halpin 1980; Sayigh et al. 1999), reptiles (Olsson 1994), and even invertebrates (Tibbetts and Dale 2004).

Fascinating individual phenotypic variation occurs in recently fledged red-legged shags (*Phalacrocorax gaimardi*) that show striking variation in the extent of filoplumes (i.e. hair-like feathers) on the neck, plumage colour, feet colour and gular pouch colour. In this species, parents must relocate recently fledged young in crèches, and so the phenotypic variation is possibly the outcome from selection for identity signals (Rasmussen 1988). A famous example of acoustic recognition is found in king penguins (*Aptenodytes patagonicus*), which are able to recognize their chick after weeks, or even month, of separation within a huge crèche of conspecific chicks (Aubin and Jouventin 1998). In colonially nesting bank swallows (*Riparia riparia*) Beecher et al.(1981a; 1981b) provided data showing that parents can identify their offspring from their begging calls. These studies indicate that individual recognition might be important during the post-fledging phase when chicks are still nutritionally dependent on parental provisioning. Selection might therefore promote identity traits in signals important in parent-offspring communication. Offspring begging calls are also known to be the important signals of need in parent-offspring communication (Kilner and Johnstone 1997). The combination of dynamic signals of need and expected phenotypic stable identity traits leads to a very interesting multicomponent signalling system revealing different aspects of an individuals state and origin (Candolin 2003).

While almost all studies that focused on parent-offspring recognition have shown that a chick can acoustically identify its parents, e.g. in penguins and gulls (Charrier et al. 2001; Searby et al. 2004), little evidence exists about the mechanisms how parents are able to identify their own offspring (McArthur 1982; Lessells et al. 1991; Medvin et al. 1993). Parent-offspring communication offers the unique possibility to investigate experimentally the developmental mechanisms and signalling function of a multi-component signal and the recognition mechanisms by both parent birds and their fledglings. For my doctoral research I was specifically interested in four aspects of the individual recognition system in parent-offspring communication:

1. How is individuality encoded when concurring signals (i.e. condition dependent hunger information and static identity information) are expected in vocalizations of nestlings?
2. Are signals of individual identity and brood identity determined by genes, environment or both?
3. Do parents and their offspring show mutual recognition?
4. Is recognition based on acoustic similarity; a prerequisite for negative-frequency dependent selection?

In the following I am going to provide relevant information on different aspects of identity signalling systems, highlight the questions I aim to answer and present the different studies I conducted.

How are individual signatures generated and perceived?

Identity signals can occur in very different sensory modalities and include chemical, visual and acoustic signals. They are often used in sensory pathways that are largely important for other life history traits such as feeding or reproductive behaviour. For example in most species of birds, olfaction is considered to be less important than visual or auditory cues. However, several tubenose species (*Procellariiformes*) that largely rely on olfaction to find food patches in the open ocean (e.g. Hutchison and Wenzel 1980) also show the ability to readily identify their nesting burrows by odour (e.g. Bonadonna et al. 2003; Bonadonna et al. 2004). In analogy, it is therefore not surprising to find acoustic identity traits to be important for many bird species as vocalizations have been shown to be a major tool in a broad variety of social interactions (Kroodsma et al. 1996).

From a signaller's perspective, the most important part of the identity signalling system is the signal that is produced. This signal must be perceivable by the receiver and provide sufficient individual distinctiveness to allow reliable recognition. Frequency modulation (FM) has been suggested to be a very important acoustic characteristic for identity recognition in birds. In a sonographic representation (for an example see chapter 1, figure 1.2), FM is the pattern that is created by the changes in frequency over time. The visual representation could almost be described as the unique acoustic 'hand writing' of an individual and is appropriately referred to as an 'individual signature'. Playback experiments on king penguins have demonstrated that the FM pattern of a call tells a chick when its parents are back from a feeding trip (Jouventin et al. 1999). In chapter 1 of my dissertation I exam whether FM is affected by changes in hunger of a chick and in chapter 5 I test if FM, next to other parameters, is determined by the genetic origin of a chick or whether the rearing environment is affecting a chicks individual signature.

Phenotypic variation used as identity signals is thought to not only represent random variation but be a result of selection for distinctiveness (Tibbetts and Dale 2007). Comparative studies on acoustic parent-offspring recognition, especially by excellent research on swallows (Medvin et al. 1993) and penguins (Jouventin and Aubin 2002), suggest that the extend of variability in identity signals is positively correlated to the degree of coloniality in a species. The selective mechanism producing an increase in phenotypic variation is thought to be mainly negative-frequency dependent, meaning that the rarer a specific phenotype of a signaller, the higher its fitness. Different to directional and stabilizing selection, negative-frequency dependent selection has the potential to actively increase the phenotypic variation in a population (Figure I.1).

To illustrate how negative-frequency dependent selection acts, I chose egg coloration in inter-specific nest parasitism as a hypothetical but intuitive paradigm. Assuming a population of birds that is parasitized by a cuckoo and both host and cuckoo exclusively lay green eggs. At some point one female is laying red instead of green eggs. The differing egg colour is enabling the female to reject green eggs laid by cuckoos and therewith giving it a selective advantage. Eventually, a cuckoo morph with red eggs will also spread, eliminating the selective advantage of red eggs over green ones. As a consequence, a new host egg colour could spread, and so on. Negative-frequency dependent selection is a very dynamic process in which selective advantage dependent on the frequency of a morph within a population; the rarer the higher the benefits. As a morph becomes more common, the benefits diminish. This selective mechanism therefore has the potential to evolve distinct phenotypic variation within a population (Tibbetts and Dale 2007).

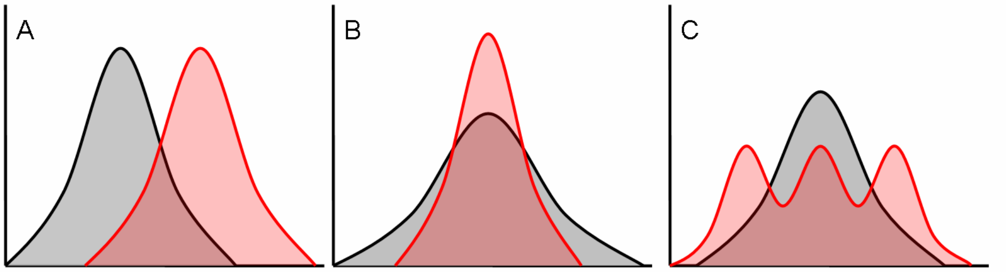

Figure I.1
Illustration of the evolution of trait properties under different selection types. The black line indicates trait properties before and the red line after selection. A) A directional shift in trait properties as a result of directional selection. B) Reduced variation due to stabilizing selection. C) Multimodal trait properties as a result of negative-frequency dependent selection.

A different mechanism that is able to create variation in acoustic traits is learning. It is well established that songbirds learn their species-specific songs from conspecifics. For example, skylarks (*Alauda arvensis*) of one area share a common dialect and are able to discriminate between members of different populations by their songs (Briefer et al. 2008). These songs are learned from each other by imitation and each population has, like in humans, its own dialects. These dialects are a result of a cultural transmission of population specific dialects (Slater 1986). The same learning mechanism also acts on the family level in so species. In long-tailed tits (*Aegithalos caudatus*), which is a cooperative breeder, young learn the family's common contact call from its family members (Sharp et al. 2005; Sharp and Hatchwell 2006).

These two mechanisms, heritable vocalizations and learned calls or songs, can even be found in the same species. In zebra finches the contact calls of females are heritable whereas the calls of males are learned from a tutor (Forstmeier et al. 2009). The heritable component is thought to mainly determine the morphology of the vocal tract, whereas the learned component reflects the content of a vocalization. It is therefore counter-intuitive to pin point the source of individual identity in a bird's contact or begging call. In chapter 5, I provide insight into the determination of identity signals. Cross-fostering experiments can be used to disentangle effects due to nest of origin (genetic effects and maternal effects) and nest of rearing (environmental effects; e.g. learning) and thus investigate its influences on the expression of acoustic identity traits (Todrank and Heth 2001). Nest of origin-related variation in begging calls mainly reflects genetic factors and non-genetic maternal effects. Nest of rearing-related variation may result from local environmental factors such as learning from other conspecifics (i.e. adults or sibling) or condition of the nestlings (e.g. food supply or nestling competition).

The other side of every signalling system is the receiver. Receivers can learn to discriminate individuals using phenotypic differences between them. In birds, studies have shown that learning of acoustic characteristics widely occurs and that this ability can be developed early in a bird's life. For example in Guillemots, young are able to memorize their parents' calls as early as a few days before hatching (Tschanz 1968). Parent birds in turn also show the ability to learn their offspring's calls (e.g. Lessells et al. 1991; Draganoiu et al. 2006) and even facial feather patterns (Stoddard and Beecher 1983) has been shown in a number of cross-foster and playback experiments.

As shown for variation in signals, also the receiver side is thought to have evolved specialized mechanisms to improve recognition of individuals in species in which an individual can benefit from this ability. The receiver can increase its perceptual sensitivity for between-individual differences to achieve recognition, either by increasing the sensorial sensitivity or the neurological processing of the signal. One striking example of an adaptation for recognition is face recognition as found in humans (for review see Kanwisher and Yovel 2006), primates (e.g. Marechal et al. 2010) other mammals (for review see Tate et al. 2006). In all these examples specialized brain areas enable an individual to identify another individual based on facial features specific to each individual. However, compared to variation in signals of identity, the abilities of a receiver to perceive and process a signal are rather difficult to investigate.

While the perceptual means might be adapted for reception of identity signals, the receiver still needs to learn the individual signatures of its social conspecifics. This process is thought to work via templates for each individual (McGregor and Krebs 1984). Is a contact call or a song perceived, the receiver tries to match the just heard call or song with the previously acquired template to identify the sender. One can imagine that, depending on the number of individuals to be distinguished, templates must be more complex with an increasing number of individuals to tell apart.

Why study identity signalling in parent-offspring communication?

Parent-offspring communication is an exciting system to study the evolution of identity signals. Firstly, because the social context is very clear. In colonially breeding species, in which parents face the risk of mistaking their own offspring for unrelated young, parents need to recognize their mobile young (Beecher et al. 1981b). Failure of recognition is expected to be very costly for the offspring and the parent, most likely leading to the death of the offspring. Secondly, parents are thought to learn the individual signatures of their chicks to obtain an acoustic template of their chicks' calls. This happens in nest breeding birds a few days before fledging when the chicks are still inside the nest. Therefore, even though individual signatures are expected to be most important after the chicks are fledged and intermingle with other offspring, their individual signature are already developed before they leave the nest (see Figure I.2).

Furthermore, parent-offspring communication is not only about identity but, also about the chick's needs and condition. Offspring begging behaviour is widely known to function as a signal of need to parents and/or siblings (Kilner and Johnstone 1997). By the costly advertisement of their state of need (e.g. hunger level or body condition), chicks allow parents to reliably allocate resources where they are most needed. Signals of need are therefore expected to exhibit large intra-individual gradual variation, directly related to body condition or hunger level of a chick (Kilner et al. 1999). In contrast to signals of need, begging characteristics that are used for recognition mechanisms are expected to show low intra-individual variation and high consistency, which facilitates recognition by its parents. Selection should therefore favour acoustic identity signals in the begging call that are not related to the chicks' physiological state and allow accurate identification, independent of hunger level and/or body condition.

Most bird species also allow easy manipulation of chick identity or condition. Cross-foster experiments at egg or early nestling stage allow to disentangle genetically determined variation from environmentally affected variation (Medvin et al. 1992; Mateo and Holmes 2004). Nestlings also allow manipulation of their body condition (e.g. hunger) to specifically test which acoustic or visual characteristics of their begging display reliable change with, and therefore encode for, the chicks body condition (Kilner and Johnstone 1997).

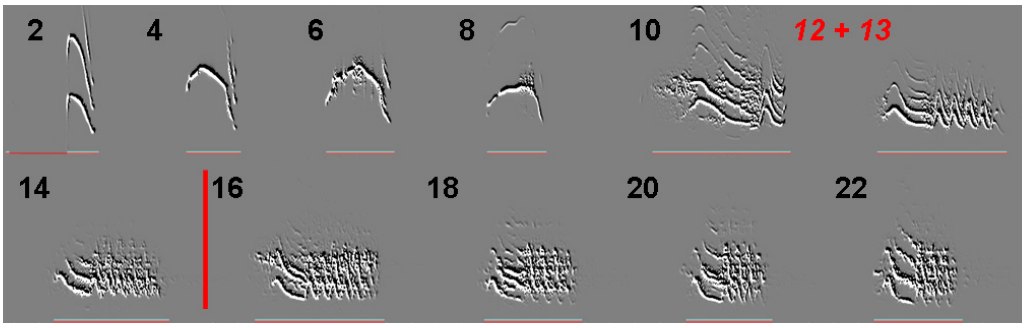

Figure I.2

Representation of the call development in Jackson Golden-backed weaver, Ploceus jacksoni, chicks from day 2 to day 22 after hatching. In red is indicated 1) the timing of the feeding experiment described in chapter 1, and 2) the timing of fledging at 16-18 days of age. Around 10 days after hatching the calls change from a pure and frequency-modulated whistle to a much more complex call that resembles contact calls of fledglings (Own unpublished data).

Study systems

For my doctoral research I worked on three different study systems. Highly colonial African weaver birds of the genus *Ploceus*, of which I focused on the Jacksons's golden-backed weaver (*Ploceus jacksoni*), provide an ideal system to study identity signals in the field (see chapter 1). In a natural colony at the shores of Lake Baringo/Kenya, I investigated how nestlings signal their hunger and their identity simultaneously. Next, to study the effects of genetic background and rearing environment on brood and identity signatures I work with tree swallow (*Tachycineta bicolor*) in collaboration with Prof. Dr. Marty Leonard and Dr. Andy Horn at Dalhousie University in Halifax/Canada (see chapter 2). The advantage of tree swallows over weaver birds when studying brood signatures is their bigger clutch size, 4-6 nestlings in tree swallows compared to 2-3 nestlings in weavers, enabling efficient cross-foster experiments. Additionally, vocalizations of nestling tree swallows are one of the best known study systems in nestling vocalizations. However, the field environment makes large scale playback experiments on mutual recognition difficult. My last study system of choice was therefore a captive population of zebra finches (*Taeniopygia guttata*) at the Max Planck Institute for Ornithology (see chapters 3-5). The controlled laboratory environment made playback experiments particularly easy.

How to analyse acoustic signals?

For insight into the characteristics and functions of acoustic identity signals, we used modern sound analysis methods that were developed for the analysis of bird vocalizations, specifically to compare details of vocalizations between individuals. Sound Analysis Pro (SAP) has been developed to study song learning in zebra finches, which requires comparisons between a young bird and the tutor it learns its song from (Tchernichovski and Mitra 2004). SAP is therefore the ideal tool to quantify fine scale differences between individuals in different acoustic dimensions. In contrast to long established methods, such as cross-correlation approaches or visual examination of sonograms, SAP uses modern computing power to employ complex algorithms and extracts detailed information for many different acoustic parameters (Figure I.3). Those measurements are a description of different physical aspects of a vocalization in different dimensions (e.g. temporal, frequency and power distribution).

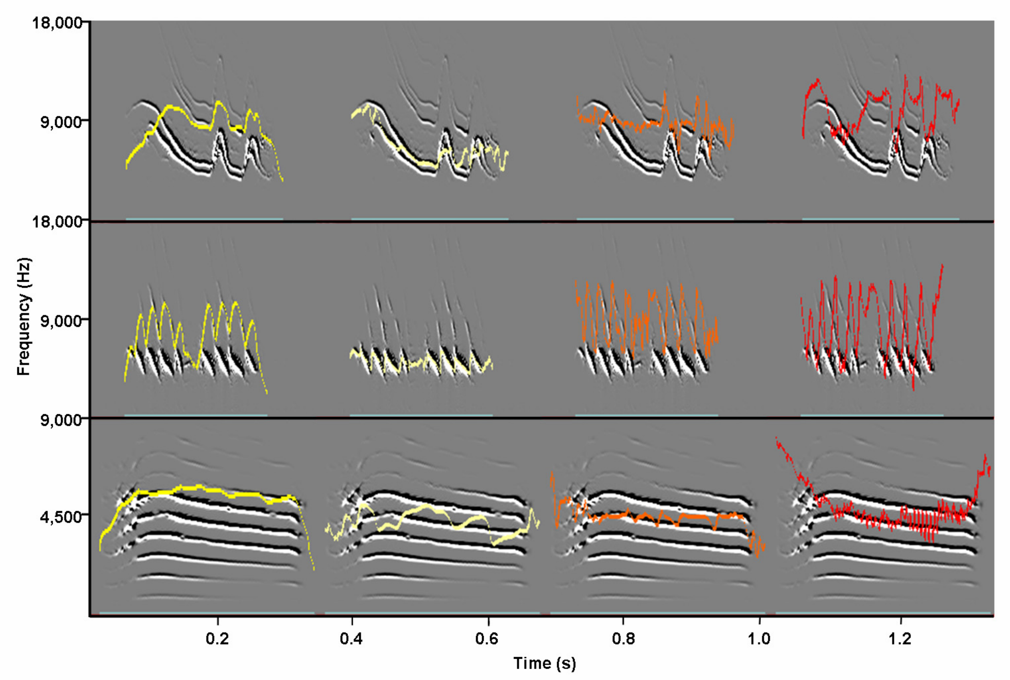

Figure I.3
Examples of call measurements from Sound Analysis Pro (SAP). The first row shows spectrograms of a weaver bird nestling call, the second row shows a call of a tree swallow nestlings and the last row shows a call of a zebra finch fledgling. The first measure in yellow is the amplitude, in cream the mean frequency is measured, orange shows the measure for amplitude modulation and lastly, red represents entropy in the calls. Measurements in SAP are taken for each millisecond, from which the mean measurement value and the measurement variance are computed. For details see the methods sections in chapter 1-5.

Outline of the dissertation

In chapter 1, we examine in a field study how begging calls incorporate both condition dependent information about the hunger of a chick and information about the chicks' identity. Begging calls are reliable signals of a chick's condition (Kilner et al. 1999), but at the same time are thought to allow parents to learn their offspring's individual acoustic signatures closely to fledging (Beecher et al. 1981a; Beecher 1988, also see figure I.2). By manipulating hunger levels of nestlings of the colonially breeding weaver birds and apportioning variation in acoustic parameters to either hunger or identity information, we were able to show that nestling's calls contain both kinds of signalling content. Furthermore our results demonstrate that identity and condition signalling are not strictly separated. This indicates a potential importance of familiarity with an individual's acoustic range to precisely estimate condition-dependent information.

In chapter 2, we ask the question after the basis of phenotypic differences between broods and individuals. Previous studies on birds suggest that genetic differences are the main source of variation in individual signatures (e.g. Medvin et al. 1992). However, other studies found that group and kin signatures can be acquired through imitation or learning (e.g. Sharp et al. 2005). In a cross-fostering experiment with semi-colonially breeding tree swallows we compared the effect of a common origin (i.e. genetic and maternal effects) with the influence of a common rearing environment (e.g. learning or development). Our results indicate that more acoustic variation is affected by the common rearing environment than by the common origin. Interestingly, acoustic parameters known to be important for individual recognition are largely influenced by the common origin of nestlings.

Chapter 3 investigates a fledgling's ability to recognize their parents. In a playback experiment with captive zebra finches we tested if fledglings are able to first recognize their parents from unrelated adults and second if the respond differently to mothers or fathers. Distance calls of zebra finches are differently acquired in both sexes, males learn their calls from a tutor whereas females calls are genetically determined (Forstmeier et al. 2009). We demonstrate that male calls are more distinctive that females calls. Our findings suggest that responses to unrelated adults may reflect recognition errors and indicate the importance of variation in identity signals for individual recognition processes in parent-offspring communication.

In chapter 4, we study the ability of parents to recognize their fledglings using the fledglings' distance calls. As demonstrated in chapter 3, fledglings are able to discriminate parents and unrelated adults acoustically (Jacot et al. 2010). Parent are able to discriminate their chicks by begging calls (Levréro et al. 2009), however, mutual recognition using distance calls has not yet been demonstrated. Our finding show that fledgling distance calls right after leaving the nest provide individual signatures that are similar to adult females in distinctiveness. However, despite the occurrence of individual distinctiveness parents fail to distinguish between their own and unrelated offspring.

Lastly, chapter 5 is based on the finding in chapter 3 and focuses on the likelihood of recognition errors in parent-offspring communication in relation to acoustic similarity between adults. In a laboratory experiment with zebra finches we tested whether fledglings respond more to calls of adults that are acoustically similar to their parents than to individuals that are dissimilar. Our results confirm this expectation and demonstrate that selection could act on distinctiveness of individuals if recognition errors are costly.

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Chapter 1

The effect of hunger on the acoustic individuality in begging calls of a colonially breeding weaver bird

Hendrik Reers, Alain Jacot

Abstract

In colonially breeding birds, the ability to discriminate between individuals is often essential. During post-fledging care, parents have to recognize their own offspring among many other unrelated chicks in the breeding colony. It is well known that fledglings and food-provisioning parents of many bird species use contact calls to convey their identity. These calls are also often used as hunger-related signals of need in young birds. Here, we investigate how such calls incorporate signals of need and at the same time act as reliable indicators of each chick's identity. In a field study, we experimentally manipulated the hunger level of colonially breeding Jackson's golden-backed weaver (Ploceus jacksoni) nestlings close to fledging and investigated its effects on acoustic call parameters. Some acoustic parameters that were related to the time-frequency pattern showed high individuality and were largely unaffected by a nestling's state of hunger. However, the majority of call parameters were significantly affected by hunger. Interestingly, most of these acoustic parameters showed both consistent changes with hunger and high between-individual differences, i.e. potential for individual recognition. The results indicate that individual recognition processes can be based on static, hunger-independent call parameters, but also on dynamic hunger-related parameters that show high individuality. Furthermore, these signal properties suggest that the assessment of signals of need can be improved if the signal value is referenced to a chick's vocal spectrum.

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Acoustic signalling in birds is a popular system in which to ask questions about the evolution of identity signalling systems and the costs and benefits of reliably communicating a sender's condition. These two different kinds of signals (i.e. identity and condition) require very different trait properties. Signals of identity need to be relatively consistent over time within individuals to reliably indicate the senders identity (for review see Tibbetts and Dale 2007). In contrast, condition signals, indicating short term changes in condition (e.g. hunger in food-dependent young), need to be plastic within an individual to reliably reflect the different conditions of a sender (e.g. Iacovides and Evans 1998; Leonard and Horn 2001). Both types of signals have been studied separately in parent-offspring communication. However, these two signals occur simultaneously in begging calls of young birds and the question arises: how can one acoustic signal manifest the need for simultaneous high and low intra-individual variation? So far, no study has investigated both types of signals and their contribution to the acoustic variability in a parent-offspring communication system.

In many colonially breeding species, parents need to discriminate their mobile chicks from other conspecific young (Beecher 1989). In most species, offspring still rely on parental care after a post-fledging phase and the accurate recognition of own offspring is important. Although the crucial time period for parent-offspring recognition is relatively short (i.e. ranging from a few days to several months), one expects selection for reliable recognition mechanism. In species in which misidentification is likely, failure of parents to recognize their offspring is prone to entail fitness costs for both, parents (i.e. reduced reproductive success) and offspring (i.e. starvation). Acoustic parent-offspring recognition has been shown in colonially breeding seabirds (e.g. Tschanz 1959; Lefevre et al. 1998; Charrier et al. 2001; Insley et al. 2003), with a special focus on penguins (e.g. Aubin and Jouventin 2002), and in fewer studies on songbirds (e.g. Draganoiu et al. 2006; Jacot et al. 2010). Frequency modulation (FM) has been found to be an important cue for acoustic individual recognition in birds. For example, king penguin (*Aptenodytes patagonicus*) chicks recognize their parents by FM patterns in their call (Jouventin et al. 1999) and zebra finches (*Taeniopygia guttata*) facilitate mate recognition by using FM cues (Vignal et al. 2008). Although these studies establish that FM is important for acoustic recognition, most likely a combination of different acoustic parameters is used by the receiver to recognize the signaller. By using multiple auditory components, individuals may increase the information content of the call, which is expected to facilitate recognition (Beecher 1989; Candolin 2003).

Many studies have also shown that begging calls incorporate information about a chick's energy requirements or body condition (i.e. signals of need) in an effort to solicit food from parents or compete with siblings (e.g. Godfray 1991; Redondo and Castro 1992; Kilner and Johnstone 1997; Wright and Leonard 2002; Kilner and Drummond 2007). These 'need' signals are highly dynamic (i.e. variable with changes in state of need) and exhibit large intra-individual gradual variation; such variation has been correlated to a chick's body condition or hunger level (e.g. Iacovides and Evans 1998; Kilner et al. 1999; Marques et al. 2009). An increase in begging intensity is often associated with an increase in call rate, call amplitude and begging bout length (reviewed in Kilner and Drummond 2007; but see Anderson et al. 2010). Compared to these characteristics of call delivery, the influence of hunger on acoustic parameters of individual calls has been studied in relatively few species (e.g. Leonard and Horn 2001; Sacchi et al. 2002; Gladbach et al. 2009; Marques et al. 2009; Anderson et al. 2010).

Consequently, in species where selection favours individual recognition, begging calls are likely to incorporate both identity cues and signals of need. Therefore, begging calls represent an excellent system to investigate the expression pattern of static (Tibbetts and Dale 2007) and dynamic traits (Kilner et al. 1999) within the same acoustic signal. In a field study with Jackson's golden-backed weavers (*Ploceus jacksoni*) we aim to identify how variation in begging calls simultaneously reflects changes in hunger and incorporates individual distinctiveness. This species is perfectly suited for studying the evolution of individual signatures in begging. It is a colony breeder with highly synchronized breeding at the start of the rainy season. This high synchronization leads to simultaneous fledging on a massive scale, in which parents must be able to locate their offspring in order to provide post-fledging parental care (e.g. food provisioning) (Fry and Keith 2004).

In a first step, we experimentally manipulated hunger levels of nestlings close to fledging and predicted that this treatment would change acoustic parameters in relation to a nestling's hunger level. The time point is important because we predict individual signatures to be developed closely before fledging to enable parents to learn individual signatures while the nestling is still in the nest. Following this, we analyzed the variance components in the begging calls to apportion the observed variance to differences between hunger levels and individuals. Our prediction was that begging calls contain stable individual information over varying hunger levels to reliably indicate a nestling's identity and dynamic information about a chicks hunger. In a last step, we used multivariate methods (i.e. discriminant function analyses) to demonstrate statistically if and how distinctiveness of individuals changes with hunger.

METHODS

Field study

This study was conducted on the western shores of Lake Baringo/Kenya (N 0°36′40″; E 36°1′20″) in the East African Rift Valley. The Jackson's golden-backed weaver is a colonial breeder with colony sizes reaching 200 nests. Breeding starts at the beginning of the rainy season (April to September), but the precise onset of breeding depends on the occurrence of the first rains and therefore varies between years. Males are polygynous and build up to five nests. Females choose nests and lay two to three eggs. The incubation period is about 14 days and nestlings hatch asynchronously because incubation starts with the first egg. In our colony, nestlings fledged 17±2 days after hatching. Most nestlings within the colony fledged very synchronized (i.e. within around a week) (own unpublished data, Fry and Keith 2004). Like other weaver species, fledglings are fed for about 2-3 weeks, in most cases exclusively by the mother (Fry and Keith 2004). Begging calls of *P. jacksoni* change during ontogeny from a simple to a complex call that shows two distinctive parts (own unpublished data). The first part of the call is whistle-like, descending in frequency, while the second call part consists of repeated elements that show an upside down U-shaped pattern in spectrograms and sounds like a short trill (Figures 1.1 and 1.2). All analyses were done separately for each call part.

We monitored nests during the breeding seasons of 2007 and 2008. Nests were checked daily during laying onset, hatching and fledging dates. Nestlings were marked individually by plucking down feathers on the head on the day of hatching and with individually numbered alloy rings on day 8. To investigate the effect of hunger-related variation on behavioural and acoustic begging characteristics, we performed a food deprivation experiment with 49 nestlings from 29 nests (1-2 nestlings/nest). Food deprivation experiments were performed shortly before fledging (2007: N=17 birds, 13±1 days old; 2008: N=32 birds, 12±0 days old). Three of those 49 nestlings did not accept manual feeding and were excluded from further analysis. Prior to the experiment, all chicks were weighed with an electronic balance (CM 150, Kern, Balingen-Frommern, Germany) to the nearest 0.1 g (mean±sd, 16.7±2.6, N=49) and tarsus length was measured with slide callipers to the nearest 0.1 mm (mean±sd, 19.4±1.1, N=49). After the experiment, a small blood sample was collected for molecular sexing (23 males, 23 females) (Griffiths et al. 1998). We were not able to collect enough blood from three nestlings for sexing.

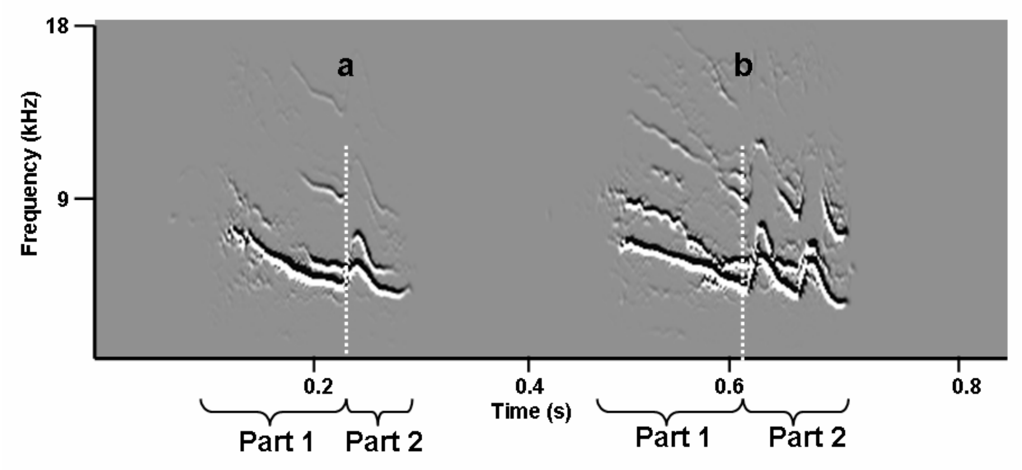

Figure 1.1
Spectrograms of representative calls of a 13 days old chick (A) 15 minutes and (B) 120 minutes after food deprivation. Note the two distinct parts of the call and the differing number of trills in the second part of the calls (i.e. one trill in (A) and two trills in (B). Spectrograms are done using SAP.

Food deprivation and recording of begging calls

In the food deprivation experiment, nestlings were temporarily removed from the natal nest and moved to an artificial nest cup, made from a plastic box (6 cm in diameter) and a cloth lining. The experimental setup was located 50-150 m from the colony at the lake shore. We placed one nestling at a time in its own artificial nest cup at a fixed position inside the experimental box (card board, 40x40x20 cm, width x depth x height). To decrease echoes and ambient noise, the box was sound-shielded on the inside with acoustic foam (N04HG, schaumstoff.com, Bochum, Germany). Prior to the experiment, nestlings were fed rearing food pellets (NutriBird C15, Versele-Lage GmbH, Wesel, Germany) until they did not accept any more food. The procedure ensured the standardization of hunger levels at the start of the experiment. During this satiation process hungry nestlings readily responded to the stimulus (see below) with begging behaviour, but became gradually less responsive with increasing satiation. After satiation, begging behaviour was induced and recorded every 15 minutes for the next two hours, starting 15 minutes after satiation (for similar protocol see: Kilner et al. 1999). Begging behaviour was induced in a standardized way by gently jolting the nest cup, simultaneously producing three consecutive soft broadband, noisy sounds with the lips and feeding one food pellet (about 0.05 g). Own preliminary studies had shown that feeding a small food pellet elicits the most repeatable begging response, while chicks were still getting hungrier. Most importantly, our treatment ensures that all chicks were fed the same number of food pellets over the experimental time period.

A microphone (C2, Behringer, Willich, Germany) was placed in a fixed position 8 cm directly above the artificial nest cup and the fledglings' calls were recorded at a sampling rate of 44.1 kHz and 16 bit amplitude resolution onto a solid state recorder (Microtrack II, M-Audio, Irwindale, USA). In order to confirm that the treatment resulted in an increase in hunger, we filmed the nestling during the experiment to quantify changes in begging posture (JVC GZ-MG77, Yokohama, Japan). Maximum begging posture was categorized into five states adapted from previous studies (Redondo and Castro 1992; Kacelnik et al. 1995; Kilner 1995): 1) no reaction; 2) opening bill, but refusing to feed; 3) acoustic begging, little wing flap, neck not stretched; 4) acoustic begging with wing flap and/or neck stretched; 5) strong acoustic begging, neck stretched all the way, standing up and flapping wings. After the experiment, the chicks were fed until satiation and placed back into their original nests. All nestlings were accepted once they were put back into the nest and no premature fledging was observed.

Sound analysis

Hunger-related variation in acoustic begging behaviour was measured using the nestling's quantitative and qualitative response. As a quantitative variable we measured call rate by counting the number of begging calls in the 10 seconds following the first begging call emitted in response to a stimulus.

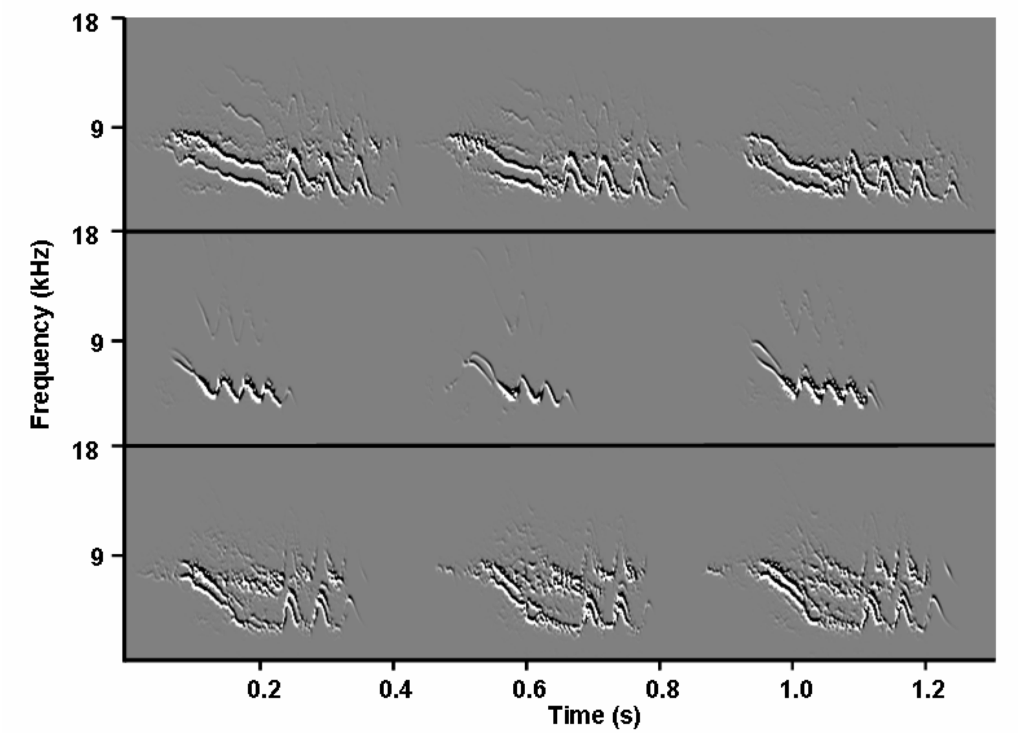

Figure 1.2
Examples of acoustic variation between- and consistency within-individual for three representative individuals. All shown calls are from individuals after 120 minutes of food deprivation. Spectrograms are done using SAP.

The qualitative response of nestlings was measured as the maximum intensification of a nestling's acoustic begging, estimated as the calls with the highest amplitude (Leonard and Horn 2001). When nestlings respond to a stimulus, their response pattern shows often a clear pattern with high-motivation calls shortly after the stimulus and a continuous decrease in motivation thereafter. Therefore, taking random calls for the analysis of a nestling's hunger-related changes in call characteristics may not capture the biologically important information. The hunger-related qualitative changes in begging call characteristics were analysed by manually selecting the five calls with the highest response to the stimulus (i.e. calls with the highest amplitude). Using a sub-sample of 23 nestlings for which we randomly selected calls we confirmed that our call selection lead to similar estimation of call parameters.

Begging calls were analysed using the computer program Sound Analysis Pro 2.063 (SAP) (for details see Tchernichovski and Mitra 2004). Compared to other methods that use visually assessed measurements from spectrograms, SAP uses complex algorithms to calculate values for each millisecond of the call and provides means and variances of those values. The begging calls of *P. jacksoni* nestlings consist of two distinct parts (Figure 1.1). For analysis we derived 10 acoustic parameters for each call part separately: 1) duration of call part (in ms); 2) mean amplitude (in dB); 3) variance of amplitude modulation (in 1/ms); 4) mean frequency (in Hz); 5) mean frequency modulation (in Hz); 6) variance of frequency modulation (in Hz); 7) mean entropy; 8) variance in entropy; 9) mean pitch (in Hz) and 10) mean pitch goodness.

The amplitude measure was not standardized between recordings of different nestlings but was consistent within nestlings. We therefore used amplitude only to measure within-individual changes. All other acoustic measures are independent of the absolute amplitude and are therefore unbiased by amplitude differences between recordings. Frequency modulation is an estimate of changes in frequency over time with high values meaning high frequency changes over time and vice-versa. Amplitude modulation is the change in amplitude over time; high values represent high changes in amplitude. Mean frequency provides a smooth estimate of the frequency with the highest power. It is calculated as mean frequency, weighted by amplitude, and therefore does not 'stick' to any frequency trace within the spectrogram. Entropy is a measure of how noisy a sound is; pure tones show low entropy, while broadband sounds show high entropy. Pitch as measured by SAP is an estimate of the fundamental frequency, based on how harmonic a sound is. For tonal sounds (e.g. a whistle) pitch is estimated as mean frequency; for harmonic sounds pitch is the fundamental frequency. The measure is weighted by pitch goodness, giving harmonic sounds more weight than tonal sounds to get a more robust measure of fundamental frequency. Pitch goodness measures the harmonic richness of a sound; low pitch goodness indicates a sound with strong harmonics while high pitch goodness indicates a pure tone without harmonics. Variances of acoustic parameters are a measure of changes over time. A high variance means high changes over time; low variance indicates little changes over time (for details see Tchernichovski and Mitra 2004).

The start and the end point of the overall call was automatically assessed in SAP by an amplitude-threshold of 25 dB and an entropy-threshold of -1.3 (for details see Tchernichovski and Mitra 2004). These values provided the best separation of calls from background noise. Calls were then manually separated into two parts by one observer. The cut-off was defined as the lowest point of the loudest frequency band, just before the repeated trill part (Figure 1.1).

Statistical analysis

General statistical analysis

All statistical analyses were performed with R 2.8.1 (R Development Core Team 2008). Variance of entropy from part 1 and 2 and variance of amplitude modulation from part 2 were log-transformed to approach normality. The effect of hunger on begging behaviour and acoustic call parameters was estimated in linear mixed effect models (LMM) (Pinheiro and Bates 2000). The potential for individual identity coding (PIC) was assessed for every acoustic parameter (Robisson et al. 1993). Finally we used a discriminant function analysis (DFA) to quantify discrimination potential for individuals in relation to hunger (package MASS, Venables and Ripley 2002).

Hunger effects on begging

The effect of hunger on begging posture, number of calls and acoustic call parameters was estimated by including year as a categorical fixed factor, hunger state (ranging from 15 – 120 minutes in steps of 15 min) as a continuous covariate and individual and nest identity as random factors. We extracted variance components from LMMs on the effect of hunger on acoustic parameters to apportion the observed variance to hunger state, nest identity and individual identity. In those LMMs, we used year as a fixed factor and hunger state, nest and individual as random effects. Sex, body mass, tarsus length and hatching order were initially included as covariates into the mixed-effects models for effects of hunger on begging posture, number of calls and acoustic parameters. Out of 88 tests, only 6 were borderline significant (range: p=0.0104–0.0472), but became not significant after adjusting the significance level to a=0.0006 using the Bonferroni method (Sokal and Rohlf 1995). Those parameters were excluded from the final models, thereby simplifying the models. The fact that neither body mass nor tarsus length had an effect on acoustic parameters is most likely due to limited variance in those variables, even across sexes. Year was included in all models to account for age (chicks were recorded at 13 days of age in 2007 and 12 days of age in 2008) and season effects. The standard model diagnostics of non-normal errors, non-constant error variance and the presence of outliers were performed on each of the final models according to Fox (2002).

Potential for individual coding

PIC is a measure of the ratio of inter-individual variation in comparison to intra-individual variation. To describe the intra- and inter-individual variations of each variable, we used the coefficient of variation (CV). For each variable we calculated CVi (intra-individual CV) and CVb (inter-individual CV) according to the formula:

$$CV = 100 \times (SD / X)$$

where SD is the sample standard deviation and X is the sample mean (Robisson et al. 1993). PIC is the ratio of CVb divided by the mean of CVi of all individuals. For a given variable, a PIC value greater than one suggests that this variable may be used for individual recognition since its intra-individual variability is smaller than its inter-individual variability. PICs were calculated for parameters both over all hunger levels pooled and for the maximum hunger level (i.e. at 120 min). PICs were not calculated for mean amplitude since amplitude was not standardized across individuals.

Discriminant function analyses

We performed two sets of DFAs to statistically investigate individuality. In the first DFA, we investigated whether calls stay individually distinctive over all hunger states. This analysis is performed without information about a chick's hunger state. A high chick assignment rate would demonstrate that a chick's voice remains distinctive independent of its hunger state. In a second set of DFAs we tested how individual discrimination is affected by changes in hunger. Here, we performed separate DFA's on four time intervals of food deprivation: i) 15 and 30; ii) 45 and 60; iii) 75 and 90; iv) 105 and 120 minutes. Creating time intervals, i.e. lumping 2 time points, was necessary because several individuals only called once during a given time point, but at least 2 calls per individual are required to calculate a cross-validated DFA (see below). Following the DFAs, we performed a linear mixed-effects model with individual as random factor and average assignment rate (four levels per individual) as continuous variable to investigate changes in individual discrimination over hunger states. For each set we conducted DFAs on both parts of the calls separately and on both parts combined. For all DFAs we used 9 call parameters (all but mean amplitude) per call part, and 18 call parameters for DFAs on both parts combined respectively. All DFAs were done using a cross-validated (leave-one-out) procedure, which fits the left out call into a multidimensional signal space computed from all calls except the one which was left out. The left-out call was then assigned with a certain probability to each individual based on the Mahalanobis distances from each call to the centroid of each individual (package: MASS, Venables and Ripley 2002).

Ethical note

This study has been approved by the Kenyan Ministry of Science and Technology and the National Museums of Kenya (permit number: MOST 13/001/38C251). We did not encounter any problem with nestling survival during or following the experiments and fledging rate was comparable to untreated nests.

RESULTS

Behavioural and acoustic response to food deprivation

Food deprivation affected both begging posture (LMM: b±SE=0.99±0.05 posture index/h, $p<0.001$, N=45 individuals) and call rate (LMM: b±SE=5.23±0.37 calls in 10sec/h, $p<0.001$, N=46 individuals). Call rate (calls/10 seconds) was nearly twice as high in hungry chicks (hunger time=120min: mean±SE, 16.7±4.7) compared to satiated chicks (hunger time =15min: mean±SE, 8.6±6.7). Neither the sex of the nestling nor its hatching order had an influence on posture or call rate (all $p>0.24$). These results demonstrate that the experiment effectively altered the hunger state of chicks, a prerequisite for investigating the static and dynamic properties of call characteristics important in signalling need and individuality.

Acoustic response to food deprivation was measured for both call parts separately (i.e. first 'whistle-like' part and second 'trill' part). Few call parameters showed clear static call properties, i.e. traits that did not change with hunger level ($p>0.05$; table 1.1). In the first call part mean frequency modulation and variance in amplitude modulation were not significantly affected by hunger (Table 1.1). Variance in entropy was not significantly affected by hunger in both call parts (Table 1.1). Additionally, in the second call part, mean frequency was not significantly affected by hunger (Table 1.1).

Most acoustic call characteristics were affected by the hunger treatment. Mean amplitude, mean entropy and mean pitch goodness increased in both call parts (Table 1.1). Calls became louder, harsher and the energy distribution became less harmonic. Other call parameters were affected in only one part of the call. Mean frequency and pitch decreased with hunger in the first call part, but were unaffected in the second part (Table 1.1). Amplitude modulation decreased and frequency modulation increased in the second part (Table 1.1). The first 'whistle' part became slightly shorter, but the second 'trill-like' part became significantly longer (see duration, table1.1). The duration of the second part is strongly correlated to the number of trill elements (LMM: b±SE=18.19±0.57 ms/trill, p<0.0001, N=31 individuals). An increase of duration in the second part of the call is therefore caused by adding more trill elements.

Table 1.1

The effect of the hunger treatment on acoustic parameters and their variance components according to the differences in time stages of the experimental treatment (Hunger), identity (ID), origin (Nest) and unexplained variance (Residual), separately calculated for both parts of the begging call. Note that variance components for ID of mean amplitude might be overestimated since the measure was not standardized across individuals.

	Acoustic parameter		Effect of hunger treatment [a]		Variance components (in %)			
			Estimate	p-value	Hunger	ID	Nest	Residual
Part 1	Duration (ms)		-4.17 ± 1.23	**< 0.001**	2.2	37.8	20.9	39.2
	Amplitude (dB)	mean	2.98 ± 0.26	**< 0.001**	11.8	30.4	17.9	39.8
	Amplitude modulation (1/ms)	variance	$(-0.02 \pm 0.08) \times 10^{-3}$	0.753	0.2	21.4	11.0	67.4
	Frequency (Hz)	mean	-239.59 ± 36.87	**< 0.001**	3.9	31.5	19.2	45.4
	Frequency modulation	mean	0.03 ± 0.34	0.926	0.4	43.7	20.9	35.0
	Frequency modulation	variance	12.57 ± 5.19	0.016	0.3	35.7	12.7	51.3
	Entropy	(log) mean	0.19 ± 0.03	**< 0.001**	3.6	22.8	37.4	36.2
	Entropy	variance	$(-0.36 \pm 10.85) \times 10^{-3}$	0.974	0.7	26.3	7.6	65.4
	Pitch (Hz)	mean	-289.59 ± 44.52	**< 0.001**	3.8	38.1	0.0	58.0
	Pitch goodness	mean	24.33 ± 3.24	**< 0.001**	5.2	31.6	12.5	50.8
Part 2	Duration (ms)		15.85 ± 0.94	**< 0.001**	18.5	50.9	4.6	26.0
	Amplitude (dB)	mean	4.58 ± 0.26	**< 0.001**	24.2	28.8	17.7	29.4
	Amplitude modulation (1/ms)	(log) variance	$(-0.27 \pm 0.06) \times 10^{-3}$	**< 0.001**	2.3	9.5	7.4	80.7
	Frequency (Hz)	mean	-32.08 ± 37.33	0.391	0.3	32.7	7.6	59.4
	Frequency modulation	mean	1.39 ± 0.32	**< 0.001**	1.1	50.6	9.6	38.7
	Frequency modulation	variance	11.94 ± 6.00	0.047	0.6	18.5	9.2	71.7
	Entropy	(log) mean	0.26 ± 0.03	**< 0.001**	6.2	26.6	21.2	46.0
	Entropy	variance	$(-1.43 \pm 0.94) \times 10^{-2}$	0.128	0.0	31.6	1.4	66.9
	Pitch (Hz)	mean	-135.20 ± 48.15	0.005	0.4	42.0	1.5	56.2
	Pitch goodness	mean	38.60 ± 3.82	**< 0.001**	7.5	17.8	30.0	44.8

[a] *Estimates for the effect of hunger are given in change per hour. Number of dF=294 for all tests. We used 1539 calls from 46 individuals and 27 nests for all tests. Bold typing*

Variance in calls in relation to hunger, individuality and nest

In the next step we estimated and compared variation due to the hunger treatment and variation due to individuality. Call parameters important for individual recognition are predicted to be both largely independent of hunger level and highly individually distinct. Mean frequency modulation fits these predictions by showing a very high individuality in both call parts and an independence from hunger level in the first part of the call (variance components, table 1.1). Interestingly, most of the other 20 acoustic parameters also showed relatively higher percentages of variation due to individual differences compared to differences in hunger level (variance components, table 1.1). Acoustic parameters could not be strictly divided into two categories (i.e. static individual variation and dynamic hunger signal) as predicted. All 20 parameters showed higher variance components for individual effects compared to hunger effects. The effect due to nest of origin (i.e. growing conditions, maternal and genetic effects) was highly variable for different acoustic parameters (ranging from 0 to 37%, table 1.1). Of the 20 parameters from both call parts, 11 parameters showed variance components larger than 10% (8 in the first part, 3 in the second part).

Individuality in calls

Complementary to the variance components for individuals, PIC values gave a second estimate of acoustic individuality. Values for PICs over all hunger levels and variance components for individuals were related ($F_{1,16}=11.60$, $R^2=0.42$, $p=0.003$, $N=18$). Six out of 18 acoustic parameters showed PIC values higher than one when PIC was calculated over all hunger states. The durations of call parts and mean frequency modulation showed PIC values higher than one in both call parts. Additionally, in the first call part mean frequency and mean entropy showed values above one. When calculated for a standardized hunger level (i.e. 105-120 min), 13 out of 18 acoustic parameters showed PIC values higher than one and showed an overall higher individuality than PIC values over all hunger levels (paired t-test; $t_{16}=6.75$, $p<0.0001$, $N=18$).

We performed two different sets of DFAs to test for acoustic differences between individuals, i.e. the probability with which a call can be assigned to an individual chick. The results of the first set of DFAs showed that although hunger changes increased, individual chicks can be statistically discriminated with correct assignment rates well above a by-chance correct assignment rate of 2.2%. DFAs using pooled acoustic parameters of both call parts showed higher assignment probabilities (71.3 % correct assignment rate) than DFAs that only included call parameters of part 1 (48.8 %) or part 2 (45.0%), .

In a second set of DFAs, we investigated whether individuality in calls increased with increasing hunger. Individual calls are highly distinctive during all hunger states (for details see figure 1.3) and correct call assignment rates increased with increasing hunger (LMM: part 1: b±SE=10.2±2.3 percent/h, $p<0.0001$; part 2: 15.6±2.1, $p<0.0001$; both parts: 16.2±2.2, $p<0.0001$; all N=46; see figure 1.3). Calls of hungry nestlings showed higher individuality compared to calls of satiated nestlings. Again, DFAs using pooled acoustic parameters of both call parts showed higher assignment probabilities than DFAs on one part only.

DISCUSSION

Overall, we experimentally showed that begging calls contain information about a nestling's hunger, and that begging calls remain statistically distinguishable (i.e. show potential for individual recognition) over changes in hunger states. Few acoustic parameters did not change with hunger level and have the potential to act as static recognition cues. However, a strict categorization into static potential identity cues and dynamic, hunger-related components was not possible for most parameters. Several dynamic traits that varied with hunger level likewise showed high individual variation and could therefore potentially function as individual recognition cues. Here, we further develop a hypothesis about how individual recognition processes can incorporate dynamic hunger-related information, and the potential consequences for the correct assessment of a chick's hunger level.

Call complexity

The structure of Jackson's golden-backed weaver nestlings' calls is highly complex and can be classified into two distinct parts: a 'whistle-like' first part and a 'trill-like' second part. This two-parted structure emerges when nestlings are around 10 days of age and remains this complex until at least 36 days of age (own unpublished data). A similar change in complexity of contact or begging calls close to fledging has been demonstrated in the chaffinch (*Fringilla coelebs*) (Wilkinson 1980) and in the bank swallow (*Riparia riparia*) (Beecher et al. 1981a; Beecher et al. 1981b). In these two species, the call increases in amplitude, becomes longer and starts showing a complex time-frequency pattern close to fledging. This increase in acoustic complexity prior to fledging is thought to facilitate recognition by allowing the parents to learn the individual signature before the nestling leaves the nest (Beecher et al. 1981a). Our results support this hypothesis. Although DFAs using differing numbers of variables are not directly comparable, the higher assignment rate of the DFAs using acoustic parameters of both call parts demonstrates that a composite call, i.e. increased complexity within a signal, provides enhanced discrimination compared to each call part alone (Figure 1.3).

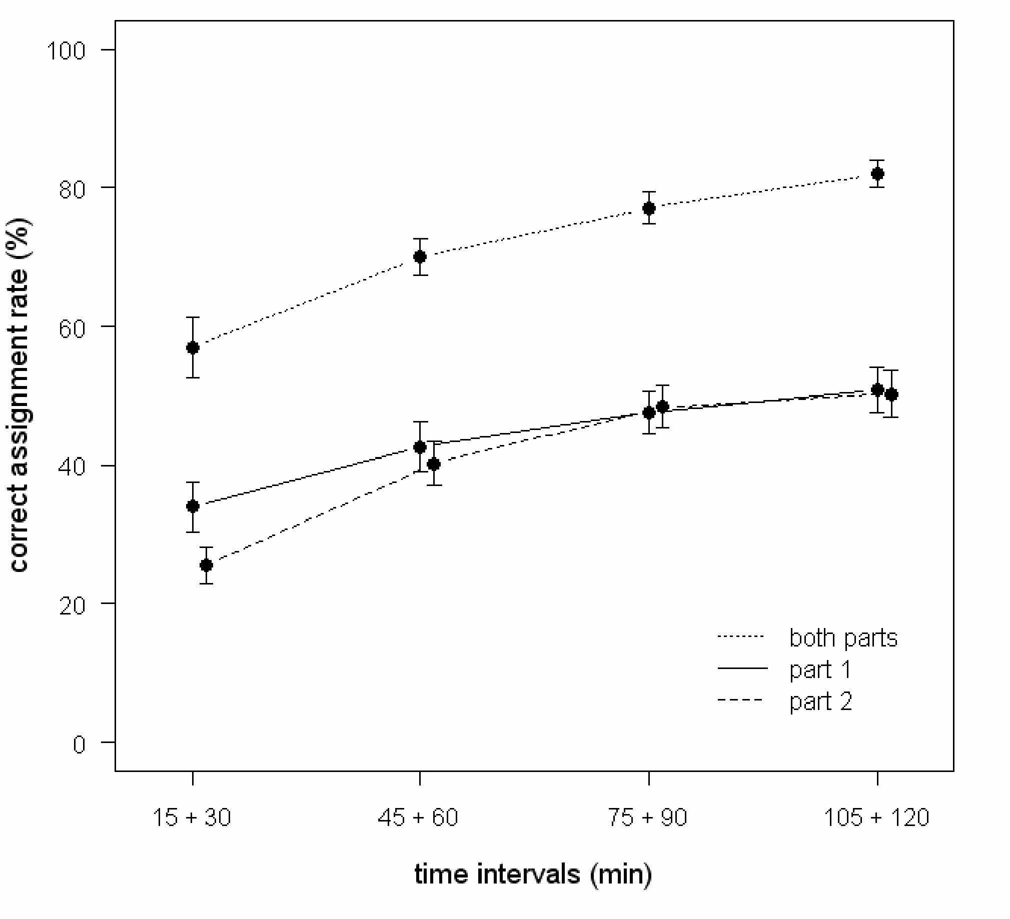

Figure 1.3

Correct assignment rates for DFA on part 1, part 2 and both parts combined on calls from four different time intervals during the treatment (mean±SE). Note that assignment rates increase with increasing hunger and the assignment rates for both parts together are almost twice as high as for single parts.

Acoustic variability within and between individuals

Most acoustic parameters in both call parts changed in response to an increase in a nestling's hunger state. 12 out of 20 parameters were strongly related to a nestling's hunger state. As predicted, amplitude increased with hunger (e.g. Iacovides and Evans 1998; Marques et al. 2009). Changes in amplitude, however, may partly be explained by decreased distances to the microphone caused by posture changes of the nestling. The second part of the call was extended by an increase in the number of trill elements. This result confirms findings on barn swallows (*Hirundo rustica*) (Sacchi et al. 2002) and tree swallows (*Tachycineta bicolour*) (Leonard and Horn 2001), showing that call duration conveys information about nestlings' hunger. These results indicate that acoustic parameters could be used by the parents to assess a nestling's need based on reliable changes with hunger. However, experimental evidence, i.e. playback experiments testing specific acoustic parameters, is necessary to test whether parents perceive and use hunger related variation as a signal of need.

Compared to the number of call parameters that were affected by a nestling's hunger, only a few parameters remained unaffected and static over all hunger levels. As found for other species, frequency modulation contained high individual variation, especially in the first part of the call. In addition, frequency modulation was not influenced by changes in hunger (Table 1.1). This suggests that frequency modulation might act as an important part of an individual signature system in Jackson's golden-backed weavers. Additionally, amplitude modulation and variance in entropy were largely unaffected by hunger in the first part of the call, while mean frequency and variance in entropy were unaffected by hunger in the second call part. However, individual recognition is most likely not based on one single component showing high individuality, but on a multitude of components and their complex interactions (Rowe 1999; Candolin 2003; Tibbetts and Dale 2007). The integration of multiple components may translate into fitness benefits due to enhanced perception of signal information (redundant signal hypothesis, Møller and Pomiankowski 1993) or due to the possibility to perceive multiple, partly independent information (multiple message hypothesis, Møller and Pomiankowski 1993). According to the variance components for individuality and the PIC values, nestling calls show potential for individual recognition in several acoustic parameters. This can enable parents to learn their nestlings' individual signature close before fledging, allowing them to relocate their offspring after leaving the nest. However, individual recognition experiments on this species are still missing. The importance of call components for potential individual recognition processes remains unknown, and needs to be addressed in future playback experiments testing the receiver's perception of differences in specific call parameters.

Acoustic individuality can also be shown when taking a multivariate approach. The DFAs over all hunger levels demonstrate that even though begging calls change with hunger, individuals can still be statistically discriminated based on acoustic parameters. Acoustic individuality is therefore maintained over changing hunger. The correct assignment rates of the DFAs increased with hunger (Figure 1.3). This finding could be expected, given that individual recognition is more important in young in high state of need. Alternatively, this finding might be due to motivational differences over the different hunger levels. Very hungry, fully motivated nestlings might produce well-stereotyped calls at maximum physiological effort, resulting in an increased acoustic variance between individuals. In contrast satiated nestlings may differ in their motivation from call to call and therefore produce less stereotyped calls which show a larger overlap between individuals.

Interestingly, most acoustic parameters showed a combination of high individuality and reliable hunger signalling. A strict categorization into static or dynamic parameters is therefore not valid. Instead, candidate cues for individual recognition also incorporate dynamic, hunger-related variation, thereby potentially signalling the nestling's hunger to the parents. For example, the duration of part 2 of the call shows clear differences between individuals (i.e. high individuality) and, simultaneously, the duration increases with hunger (Table 1.1). These signal properties raise an interesting theoretical concept about a combination of identity and condition signalling. The absolute duration of part 2 is not a reliable indicator of a chick's hunger by itself. However, when duration of part 2 is perceived on a relative scale (i.e. compared within an individual's acoustic spectrum), the perceiver can obtain a highly accurate estimate of a chick's state of need. One option is that receivers may use a general 'rule of thumb' in which they compare among several begging characteristics or relate begging characteristics to begging posture in order to assess a chick's state of need. Alternatively, receivers compare the signal with an 'individual-referenced' template of the acoustic spectrum of the sender, and thereby perceive more information about the sender's condition. In other words, by knowing the identity of the sender and being familiar with the sender's acoustic range, the receiver could improve perception of signals. While the acoustic range of the signaller may change with age, body condition or status, repeated interactions in parent-offspring communication may ensure that parents stay familiar with the acoustic spectrum of individual chicks.

Perceiving more precise acoustic information about an individual through familiarity with its acoustic signal spectrum is presumably a common, though not yet well-investigated, phenomenon. To our knowledge, very few studies on non-human animals have investigated the effect of familiarity on perceiving individuals' acoustic signals. Studies on vocalizations of great tits (Parus major) (McGregor et al. 1983; McGregor and Krebs 1984) and western meadowlarks (*Sturnella neglecta*) (McGregor and Falls 1984) found that familiarity with the songs of an individual improves ranging estimates by the receiver. McGregor and Krebs (1984) demonstrated in great tits that territory holders react differently to degraded and not-degraded songs only when they are familiar with the opponent's song. They suggested that the receiver compares a familiar song to a learned template in order to estimate the degradation of the song, and therefore cannot judge the degradation of unfamiliar songs. A very similar ranging effect has been demonstrated in humans by the same authors (McGregor et al. 1985). Further studies in humans suggest that being familiar with a specific voice increases the ability to recognize words that are overlaid with noise (Nygaard et al. 1994; Nygaard and Pisoni 1998; Burk et al. 2006). Those examples indicate that perceiving certain information requires familiarity with the signal variability of an individual. These studies highlight that individual-referenced signalling could be a widely used communication component in social communication systems, which allow learning of acoustic signal templates through repeated interactions.

Here, we used a signaller's perspective to experimentally disentangle the fine-scaled variation in multi-component begging calls of Jackson's golden-backed weaver chicks. However, to fully understand signalling systems one has to adopt a signaller's and a receiver's perspective. Thus, playback experiments are clearly needed to demonstrate whether receivers assess signal value or single call components and if those signals are assessed on an absolute scale or referenced to the acoustic spectrum of a signaller.

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Chapter 2

Determinants of nest and individual signatures in tree swallows: A cross-fostering experiment

Hendrik Reers, Alain Jacot, Andy G Horn, Marty L Leonard

Abstract

The occurrence of group and individual signatures in vocalizations has been shown in many avian taxa. However, the proximate mechanisms on how group and individual signatures arise are poorly understood. Hypotheses suggest that genetic effects (i.e. common origin), environmental effects (i.e. common rearing) or the combination of both determine acoustic signatures. In nestlings of tree swallows (Tachycineta bicolor), we investigated in a first step whether nestlings close to fledging show individual and brood signatures in their begging calls. In a second step using a non-reciprocal cross-fostering experiment, we disentangled influences due to common origin and common rearing environment on individuality and on single components of the begging calls. Taken together, the results suggest that nestlings are highly individually distinctive. In addition, brood signatures were found in unmanipulated nests, while cross-fostered nests mainly showed an effect of nest of rearing, compared to nest of origin. The strongest nest of rearing effect was on call duration, a call component known to converge in sibling competition. Frequency modulation, a trait that is important for individual recognition, was the only call parameter mainly being determined by nest of origin. These findings demonstrate experimentally that the interplay of growing environment and genetic background is important for the expression of brood signatures.

The occurrence of individual recognition using acoustic signatures has been shown in a variety of avian taxa (e.g. Stevenson et al. 1970; Lovell and Lein 2005; Vignal et al. 2008; Jacot et al. 2010). A fewer number of studies have shown that social groups, such as families or broods, use a common signature to communicate their group identity (e.g. Mammen and Nowicki 1981; Hile et al. 2000; Sharp et al. 2005; Sharp and Hatchwell 2006; Tyack 2008). Both types of signals are thought to be common in animal communication whenever there is a benefit to recognize individuals, a group or to be recognized. Such signals are known to be widespread and are important in species showing social organization (e.g. Price 1999; Sharp and Hatchwell 2006), whenever there is the potential problem of misidentification of offspring (e.g. Aubin and Jouventin 2002) and in neighbour-stranger discrimination (dear enemy phenomenon) (e.g. Radford 2005). However, the proximate mechanisms about how group or individual signatures are determined are less well understood.

Conceptually, there are two non-mutually exclusive explanations for the development of acoustic signatures. Signatures may be due to effects related to a common origin (i.e. genetic or maternal effects) or due to effects in relation to a common growing environment. The genetic hypothesis suggests that individuality is mainly genetically determined, selected through negative-frequency dependent selection (Tibbetts and Dale 2007; Sheehan and Tibbetts 2009; Sheehan and Tibbetts 2010). This hypothesis implies that siblings are likely to show more similar calls due to shared genes. As a result, parents may use inherited brood signatures as well as individual signatures to recognize their offspring. In vocalizations of young birds, cross-fostering studies have revealed that begging characteristics are based on effects due to a common origin (i.e. maternal or genetic effects). In young cliff swallows (*Hirundo pyrrhonota*), the nest of origin was a main determinant of call components that were related to the time-frequency pattern of begging calls (Medvin et al. (1992). Another study in nestling great tits (*Parus major*) showed that variation in begging intensity (i.e. the time spent begging) was mainly explained by nest of origin and not rearing (Kölliker et al. 2000).

On the other hand, variation in individual and group signatures may be related to the environment during ontogeny. Offspring within a nest share similar rearing conditions which may impact the development of the acoustic signal. This effect may either be due to similar condition-dependent developmental trajectories of calls (e.g. Leonard et al. 2000) or due to learning-based convergence of siblings' calls to each other (e.g. Sharp and Hatchwell 2006; Leonard et al. 2009; Dreiss et al. in press). Especially the work on long-tailed tits (*Aegithalos caudatus*), a social bird living in kin groups and showing cooperative breeding behaviour, has provided important evidence for learned group signatures (Sharp et al. 2005; Sharp and Hatchwell 2006). In respect to begging calls, studies on barn owls (*Tyto alba*) has shown (Roulin et al. 2009) and tree swallows (*Tachycineta bicolour*) (Leonard et al. 2009) have shown that call duration is converging between siblings under sibling-competition compared to calling alone.

The complexity of begging calls and previous evidence suggest that individual and group signatures are unlikely to be based on either common origin or common environment alone. Medvin et al. (1992) in their work on cliff swallows stated that the genetic and imitation hypotheses of sibling-sibling call similarity are distinct but not mutually exclusive. Surprisingly, so far no study has examined whether a combination of these two hypotheses exists for the development of brood signatures. A cross-fostering experiment is the ideal tool to give insight into whether similarities between siblings are based on common genes or a common environment.

In the present study we cross-fostered broods of tree swallows to investigate (1) whether siblings share distinctive individual and brood signatures and (2) whether these signatures are based on common origin or a common rearing environment. Comparative studies on swallows suggest that recognition is related to colonial breeding (Beecher et al. 1986; Medvin et al. 1992). Tree swallows breed in loose colonies and are therefore at an intermediate position on the solitary-colonial continuum, providing potential for the importance of parent-offspring recognition after fledging (Leonard et al. 1997). Nestling close to fledging (i.e. 17-20 days of age) start calling while sitting singly in the entrance hole of the nestbox, waiting for the next parental feeding visit. This behaviour of nestlings allows recordings just before fledging under natural conditions, individually and without handling. In barn swallows (*Hirundo rustica*) and cliff swallows, calls at around 17 days of age are at a 'crystallized' stage and do not change until fledging (Medvin et al. 1993). Tree swallows are similar in this regard and the calls of young given at this age are nearly identical with contact calls of fledglings used in parent-offspring communication. In a first step we recorded and analyzed calls of unmanipulated nests to investigate which call parameters contribute to brood signatures in nestlings' calls. In a second step we analyzed calls of cross-fostered nestlings to investigate how variation in those parameters is apportioned to a common environment (i.e. nest of rearing) and common origin (i.e. genetic or maternal).

METHODS

Study species

We conducted this study in the Gaspereau Valley of Nova Scotia, Canada (45° 4.5' N, 64° 20.0' W) between May and July 2010 (study sites described in detail in Leonard and Horn 1996), using a population of tree swallows nesting in nestboxes tree swallows. Nestling age was determined by checking nestboxes daily around the anticipated hatching date (hatch day = day 1). For molecular sexing we took a small blood sample on day 13 after hatching (Griffiths et al. 1998).

Cross-fostering and recording

For this study, we used a total of 18 nests, 6 control nests and 12 cross-foster nests. We cross-fostered nestlings at 3-4 days of age with a maximum of one day age difference between nests. Nestlings were cross-fostered within a triplet of nests in a nonreciprocal way with all nestlings growing up in foster nests (i.e. nestlings from nest A were divided between nests B and C, and so on, see figure 2.1) (Mateo and Holmes 2004). This cross-foster design allows separating origin and environmental effects since no nestlings grows up in its maternal nest but in a different foster nest (Kölliker et al. 2000; Mateo and Holmes 2004). A partial cross-fostering in comparison does not allow separating origin and environment since some individuals are growing up in their maternal nest; therefore origin and environment are undistinguishable (Mateo and Holmes 2004). Within each triplet, all nestlings were marked with a unique nest colour and individual colour combination by dyeing the toes with permanent marking pens (Lumicolor, Staedler, Nürnberg, Germany). To minimize nestling size differences within foster nests, which might be caused by potential size differences between the respective nests of origin, nestlings were ranked for weight within their nest of origin and assigned to foster nests alternately. Nestlings from control nests received the identical treatment, apart from the swap between nests. When nestlings were about 13 days old (mean±sd:13.0±0.4, range: 12-14), we weighted the nestlings to the nearest 0.1g (mean±sd: 22.6±1.8, range: 16.3-25.7) and measured wing length to the nearest 1mm (mean±sd: 57.1±5.2, range: 41-68). To visually identify nestlings that were calling at the nestbox entrance, all nestlings of a nestbox were marked individually by dyeing the white throat feathers using the same permanent markers as for the toes.

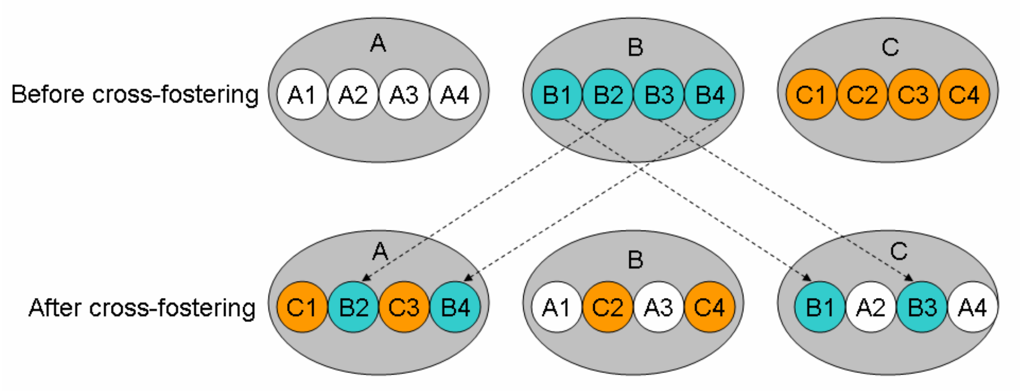

Figure 2.1:
Scheme of the non-reciprocal cross-foster design. All nestlings grow up in foster nests. Nestlings are ranked by weight, which is a proxy for age, and distributed to foster nests alternately.

Nestlings were recorded shortly before fledging (mean±sd: 19.4±0.8 days of age; range: 18-21 days). A few days before fledgling, nestlings wait inside the entrance hole of the nestbox for parental feeding visits. While being at the entrance of the nestbox, nestlings utter their contact call, even in the absence of parents (personal observations). Recordings were made with a shotgun microphone (Sennheiser ME66, Wennebostel, Germany), connected to a solid state recorder (Microtrack II, M-Audio, Irwindale, USA) and mounted on a tripod. The microphone was placed 1.5-2m from the nestbox entrance beneath the nest box, at approximately 45 degree angle, facing the front of the nestbox.

The individual occupying the position at the entrance of the nestbox was identified from 30-40 meters distance using a spotting scope.

Sound analysis

We were able to record 60 nestlings from 16 nests. Of the 12 nests that were initially cross-fostered, 2 nests were lost to predation, one in each of two cross-foster triplets. However, through the nonreciprocal cross-foster design, nestlings from all 12 nests of origin were recorded. We used up to a maximum of 25 calls per individual (mean±sd: 10.2±5.8, range: 1-25) which had to be free of any sounds other than the focal nestling. In cases in which we managed to record more than 25 calls per individual, calls for analysis were selected randomly. Calls were analysed using the computer program Sound Analysis Pro 2.063 (SAP) (for details see Tchernichovski and Mitra 2004). Compared to other methods that use visually assessed measurements from spectrograms, SAP uses complex algorithms to calculate values for each millisecond of the call and provides means and variances of those values. For analysis we derived 9 acoustic parameters for each call: 1) duration of call part (in ms); 2) variance of amplitude modulation (in 1/ms); 3) mean frequency (in Hz); 4) mean frequency modulation (in Hz); 5) variance of frequency modulation (in Hz); 6) mean entropy; 7) variance in entropy; 8) mean pitch (in Hz) and 9) mean pitch goodness. The start and the end point of a call was automatically assessed in SAP by an amplitude-threshold of 25 dB and an entropy-threshold of -3.0 (for details see Tchernichovski and Mitra 2004).

Amplitude was not standardized between recordings of different nests; we therefore did not use amplitude as an acoustic parameter in any analysis. All other acoustic measures are independent of the absolute amplitude and are therefore unbiased by amplitude differences between recordings. Frequency modulation is an estimate of changes in frequency over time with high values meaning high frequency changes over time and vice-versa. Amplitude modulation is the change in amplitude over time; high values represent high changes in amplitude. Mean frequency provides a smooth estimate of the frequency with the highest power. It is calculated as mean frequency, weighted by amplitude, and therefore does not ‘stick’ to any frequency trace within the spectrogram. Entropy is a measure of how noisy a sound is; pure tones show low entropy, while broadband sounds show high entropy. Pitch as measured by SAP is an estimate of the fundamental frequency, based on how harmonic a sound is. For tonal sounds (e.g. a whistle) pitch is estimated as mean frequency; for harmonic sounds pitch is the fundamental frequency. The measure is weighted by pitch goodness, giving harmonic sounds more weight than tonal sounds to get a more robust measure of fundamental frequency. Pitch goodness measures the harmonic richness of a sound; low pitch goodness indicates a sound with strong harmonics while high pitch goodness indicates a pure tone without harmonics. Variances of acoustic parameters are a measure of changes over time. A high variance means high changes over time; low variance indicates little changes over time (for details see Tchernichovski and Mitra 2004).

General statistics

All statistical analyses were performed with R 2.10.1 (R Development Core Team 2010).

DFA analysis

To quantify individual differences, we used discriminant function analysis (DFA) to investigate whether individuals can be discriminated statistically, based on their calls. From six nestlings we only recorded one call; we therefore excluded those from the DFA since we needed at least two calls per individual for the leave-one-out method. Therefore we used 604 calls from 54 nestlings for the DFA on individuality. To quantify whether broods can be discriminated and whether such discrimination is due to nest of rearing or nest of origin effects, we used a DFA on broods, with means of call parameters for each individual. For the DFA on nest effect we used 22 nestlings from 6 nests for the control nests. The effect of rearing and origin was done with 38 chicks from 10 rearing nests of which we used 37 chicks from 11 nests of origin, respectively. One nest of origin could not be used since we only recorded one chick of this nest. To quantify if sexes are acoustically distinctive, we performed a DFA on sex (31 females, 29 males) by using the means of each acoustic parameter for each individual. For all DFAs we used all 9 call parameters. All DFAs were done using a cross-validated (leave-one-out) procedure, which fits the left out call into a multidimensional signal space computed from all calls except the one which was left out. The left-out call is then assigned with a certain probability to each individual based on the Mahalanobis distances from each call to the centroid of each individual (package: MASS, Venables and Ripley 2002).

Variance components

To apportion the acoustic variation in call parameters either to differences between individuals or to differences in nest of rearing or nest of origin, we extracted variance components from *glms*. In those *glms*, we used sex, body mass, wing length and age of nestlings at the day of marking (i.e. around day13) as fixed factors and individual, nest of rearing and nest of origin (for cross-fostered nests only) as random effects.

RESULTS

DFA results

The DFA on differences between individuals revealed that individuals were acoustically highly distinctive and could be assigned to the correct individual statistically. 70.5% of all calls (426 out of 604 calls from 54 individuals) got assigned to the correct individual, which was significantly different compared to a 1.9% by chance probability (binomial test, $p<0.01$, for example calls see figure 2.2).

To quantify whether nestlings of one brood share a common brood signature and if this signature is depending on a rearing or an origin effect, we conducted DFAs on control and cross-fostered nests. Control nests showed brood distinctiveness that allows for acoustic discrimination. 14 out of 22 nestlings from 6 nests were assigned to the correct nest, which was significantly different with 63.6% compared to a 16.7% by chance assignment (binomial test, $p<0.01$). For cross-fostered nests, nestlings were distinguishable by nest of rearing; 9 out of 38 nestlings were assigned to the correct nest (23.7% compared to 10% by chance; binomial test, $p=0.01$). However, the assignment rate of 9.1% to the correct nest of origin did not differ from a 10.5% chance of correct assignment (4 out of 37 nestlings from 11 nests, binomial test, p=0.79).

The sexes were not distinguishable using DFA analysis. 34 out of 60 nestlings were assigned to the correct sex, which represents 57.7% of nestlings and did not differ from a by chance correct assignment of 50.0% (binomial test, p=0.37).

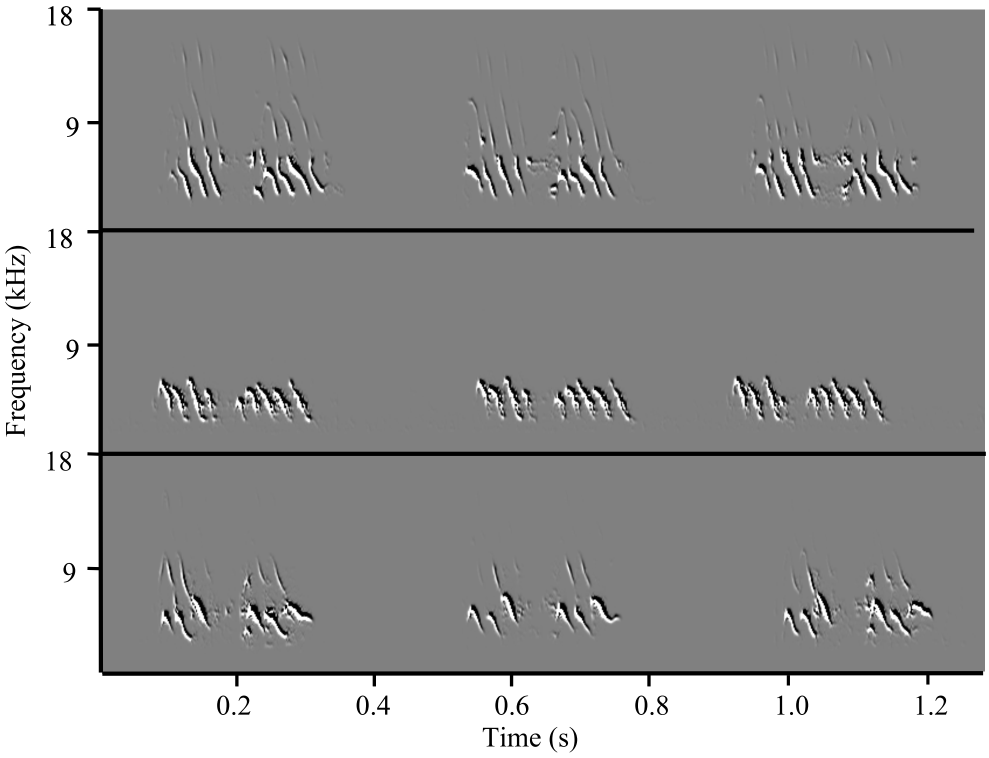

Figure 2.2:
Examples of acoustic variation between- and consistency within-individual for three representative individuals. Spectrograms are done using SAP.

Acoustic variation in individual and brood signatures

The variance component analysis in control nests showed that nestling identity explained high proportions of variation in call parameters. All but two acoustic parameters (i.e. variance in amplitude modulation and variance in frequency modulation) showed high proportions due to between individual differences (Table 2.1). In cross-fostered nests, variance components for between individual differences were on average higher than in control nests. In all analyses, nestling identity explained more than 20% of variation in every acoustic parameter (range: 20-73%, table 2.2, figure 2.3).

In a next step, we analyze brood signatures in control and in cross-fostered nests. As the DFA analysis in control nests had shown, the nestlings of one brood shared a common brood signature. For control nests, the variance component analysis revealed that all but three parameters (i.e. variance in amplitude modulation, variance in entropy and mean pitch goodness) contributed considerably (i.e. over 20% variation explained) to brood signature (Table 2.1). The cross-foster experiment allows disentangling the observed variation in control nests into effects due to common environment or common origin. Nest of rearing explained variation in call duration and variance in entropy of more than 20% (Table 2.2, figure 2.3). Other parameters did not contribute to a variation among nests of rearing. In contrast, nest of origin explained 21% of the variation in mean frequency modulation (Table 2.2, figure 2.3). Nest of origin did not explain considerable variation in any other call parameter.

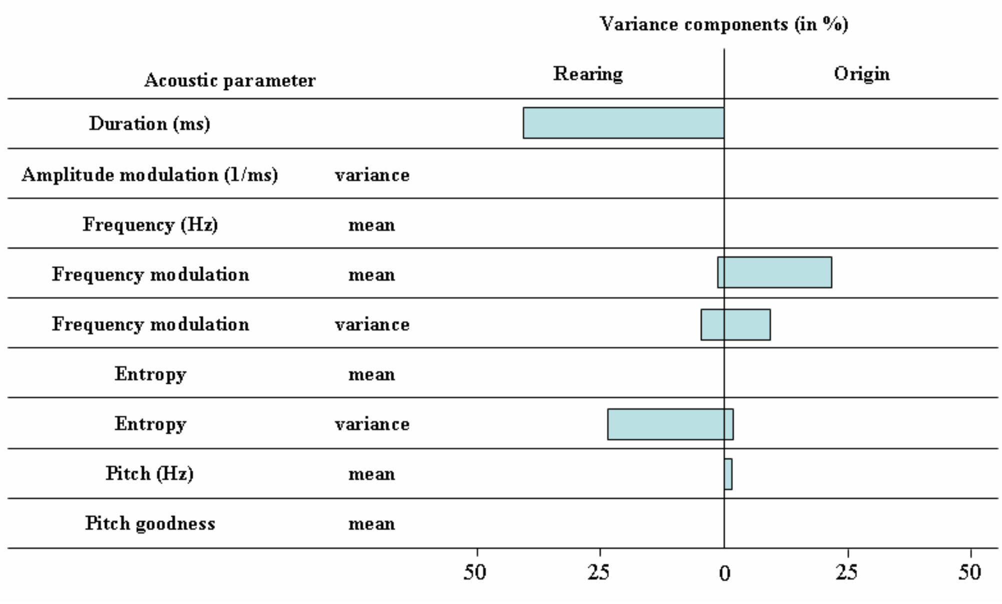

Figure 2.3:
Percentage of variance in call characteristics apportioned to nest of rearing and nest of origin.

Table 2.1:
Variance components for control nests

Acoustic parameter		Variance components (in %)		
		ID	Nest	Residual
Duration (ms)		0.37	0.34	0.29
Amplitude modulation (1/ms)	variance	0.10	0.13	0.77
Frequency (Hz)	mean	0.48	0.32	0.21
Frequency modulation	mean	0.45	0.32	0.24
Frequency modulation	variance	0.08	0.28	0.64
Entropy	(log) mean	0.24	0.45	0.31
Entropy	variance	0.44	0.14	0.43
Pitch (Hz)	mean	0.34	0.46	0.21
Pitch goodness	mean	0.45	0.00	0.55

Table 2.2:
Variance components for cross-fostered nests

Acoustic parameter		Variance components (in %)			
		ID	Rearing	Origin	Residual
Duration (ms)		0.23	0.41	0.00	0.36
Amplitude modulation (1/ms)	variance	0.20	0.00	0.00	0.80
Frequency (Hz)	mean	0.73	0.00	0.00	0.28
Frequency modulation	mean	0.52	0.02	0.21	0.26
Frequency modulation	variance	0.44	0.05	0.09	0.43
Entropy	(log) mean	0.47	0.00	0.00	0.53
Entropy	variance	0.29	0.25	0.03	0.43
Pitch (Hz)	mean	0.69	0.00	0.03	0.29
Pitch goodness	mean	0.57	0.00	0.00	0.43

DISCUSSION

This study demonstrates that tree swallow nestlings are individually distinctive close to fledging. More importantly, we found that nestlings in untreated control nests showed brood signatures. In comparison, nestlings in cross-fostered nests are only statistically distinguishable for their nest of rearing but not for their common origin. Apportioning variation in call parameters to nest of rearing and nest of origin revealed that nest of rearing mainly explained variation in call duration while the only parameter related to the nest of origin was frequency modulation, an often cited call component for individual recognition.

Individuality in nestlings calls

Nestling calls showed a high degree of acoustic individuality, potentially allowing individual recognition by their parents. The DFA on individual differences revealed that nestlings are highly distinctive, 70.5% of all calls were assigned to the correct individual. This result indicates that calls might be important for individual recognition, but experimental evidence for recognition is still missing. The relatively high rate of individuality supports the hypothesis evolution of identity signals in relation to coloniality (Medvin et al. 1993; Aubin and Jouventin 2002; Jouventin and Aubin 2002). Tree swallows show semi-colonial breeding behaviour and are therefore likely to use calls for parent-offspring recognition (Beecher et al. 1986). In both cross-fostered and control nests, the variance component analysis revealed that basically all parameters show high individuality and could be used for individual recognition.

Brood signatures in nestlings

In our study, control broods showed clear brood signatures and broods could be statistically discriminated using DFA. This finding was confirmed by the variance component analysis which revealed that considerable variation was appointed to all acoustic parameters but pitch goodness. These findings coincide with a study on cliff swallows showing that 82% of nestlings were assigned to the correct brood (Medvin et al. 1992).

In the cross-foster experiment the assignment rate of nestlings to the correct nest was lower than for control nests. This result can be predicted because the combined effects of a common rearing environment (i.e. growing conditions) and shared genes in control nests will produce more similar begging calls compared to cross-fostered nests. In cross-fostered nests the effect of brood of rearing was stronger than brood of origin. This finding indicates that brood signatures found in control nests are largely determined by the rearing environment during the nestling phases. However, not all call parameters were strongly influenced by nest of rearing. Interestingly, few call parameters were strongly influenced by the nest of origin, but almost not by the nest of rearing. The differential effect of nest of rearing and origin on call parameters highlights the complexity of contact calls

Nest of rearing effect in cross-fostered nests

The main call parameter influenced by nest of rearing was call duration (see table 2.2 and figure 2.3) for which nestling within a nest produce calls of similar length. One possibility is that similarities within a nest are due to the condition dependent development of nestlings and their voice (Leonard et al. 2000). Alternatively, this similarity in call duration within a nest could be the result of call convergence. Leonard et al. (2009) have previously demonstrated that nestling tree swallows adjust there voice when begging with another sibling. Repeated interactions among nestlings during the nestlings phase might translate into a shared brood signature. This idea is also supported by findings of Roulin et al. (2009), showing that siblings in barn owls adjust call duration to each other in the course of sibling negotiations. In both studies however, convergence in call duration between siblings is discussed as a consequence of sibling competition and not as a brood signature which might be important for recognition processes. Our results highlight the possibility that convergence can shape calls of nestlings and shows that social influences might be important for nestlings' signalling.

Nest of origin effect

The only parameter related to common origin in cross-fostered nestlings was frequency modulation. In addition, frequency modulation was largely unaffected by nest of rearing. This call component is likely to contain a genetic or maternal component, supporting the genetic hypothesis for this specific parameter. This finding is interesting since it coincides with previous studies showing that frequency modulation is an important call parameter for individual recognition in zebra finches (e.g. Lengagne et al. 2001; Jacot et al. 2010), penguins (Lengagne et al. 2000) and even in seals (Charrier et al. 2001). Since individual distinctiveness is thought to be translated into sibling-sibling similarity via shared genes, this finding gives indirect support for the importance of frequency modulation for recognition processes. Our findings are in line with models for the spread of individual signatures via negative-frequency dependent selection, for which heritability is a prerequisite.

Our finding is in contrast to a previous cross-fostering experiment in cliff swallows where all call parameters were largely determined by nest of rearing

(Medvin et al. 1992). However, given that their cross-fostering experiment took place at the egg stage they were not able to retrieve information about the nest of origin. Our study can fill this gap by measuring similarities between both genetic siblings that grew up in different foster nests and foster siblings. We showed in the DFA and the variance component analysis that there was very little genetic determination in calls of nestlings.

Here we show that signatures exist on the level of the individual and the brood. Demonstrating such signatures does not imply that variation in contact calls is used for identity recognition, both on the level of the individual and the brood. While there is evidence for individual recognition, the use or perception of brood signatures is more questionable. Brood signatures were mainly determined by the rearing environment. This acoustic similarity within a brood may largely be shaped by the intensity of sibling competition which translates into differential convergence of contact calls within a brood. The complexity of these calls highlights the importance of future research in signal perception as well as in the ultimate mechanism explaining variation in signal components.

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Chapter 3

Individual recognition and potential recognition errors in parent-offspring communication

Alain Jacot, Hendrik Reers, Wolfgang Forstmeier

Abstract

The recognition of food-provisioning parents is crucial for fledglings of many bird species. Vocalisations are the most commonly used cues in avian parent-offspring communication and it has been shown in several species that fledglings respond specifically to their parents' contact calls. However, fledglings occasionally also react to unrelated adults. Such responses may reflect recognition errors or alternatively a strategy of fledglings to obtain food or other direct benefits from unrelated adult birds. In a playback experiment we tested whether zebra finch (Taeniopygia guttata) fledglings perceive variation in adult call signatures to recognize their parents, and whether the propensity to respond to unrelated individuals is related to the gender of adults and to signal properties of male and female calls. Male calls are learnt and show high intra-sexual variation, which may improve the accurate recognition of the father's individual signature. In contrast, calls of adult females are innate, show lower intra-sexual variation such that the mother's call is more likely to be confused with another female call. We demonstrate that fledglings are able to recognize their parents. In addition, fledglings reacted more strongly to unrelated females compared to unrelated males. Our findings suggest that responses to unrelated adults may reflect recognition errors and indicate the importance of variation in identity signals for individual recognition processes in parent-offspring communication.

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In any animal species parents provide resources to their offspring before independence (Clutton-Brock 1991). By providing parental care, parents invest time and energy into their progeny thereby increasing their own fitness via producing viable offspring. From an offspring's perspective, the amount of resources they receive from their parents positively affects their survival (e.g. Perrins 1965), and the likelihood to reproduce and recruit into the breeding population (Tinbergen and Boerlijst 1990; Both et al. 1999). The importance of parental care for the fitness of offspring has lead to accurate recognition systems where fledglings use specific cues to recognize their parents.

Vocalizations are commonly used recognition cues in birds since they can be used over long distances (e.g. Charrier et al. 2001) and when visual contact is restricted. Many chicks of colonially breeding species like penguins (Jouventin and Aubin 2002; Searby et al. 2004; Searby and Jouventin 2005), murres (Jones et al. 1987; Lefevre et al. 1998) and swallows (Medvin and Beecher 1986; Medvin et al. 1992; Medvin et al. 1993; Leonard et al. 1997) use acoustic cues to identify their parents. Such recognition systems are especially likely to evolve where offspring could potentially confuse their own mother and father with other food-provisioning parents. This is the case in large breeding colonies and in species showing extended post-fledging care where parents cannot any longer use spatial cues, i.e. the nest, to locate their brood (Tibbetts and Dale 2007). Recognition cues may also evolve whenever parents show specific preferences in their long-term interactions with their offspring, feeding only a subset of fledglings within a brood ('brood division', reviewed in Leedman and Magrath 2003). Thus, parent-offspring recognition may involve class-level recognition (i.e. class being both parents) as well as individual recognition (i.e. individual parents). While there is ample evidence that fledglings of many species recognize their parents, it remains unexplored whether offspring actually use 'family' or individual signatures in parent-offspring communication.

Despite the evidence that fledglings recognize and specifically respond to their parents' calls, it is also known that fledglings sometimes respond to unrelated adults. These responses may reflect a strategy of fledglings in order to obtain food and other direct benefits from adult birds (Shy 1982; Sealy and Lorenzana 1997). Alternatively, responding to unrelated adults may reflect recognition errors where fledglings confuse unrelated adults with their parents. This definition of recognition error is based on studies demonstrating that parents recognize their offspring using acoustic cues (Buckley and Buckley 1972; Beecher et al. 1981a; Lessells et al. 1991; Draganoiu et al. 2006) and that responding to unrelated adults incurs costs. Adults are known to attack unrelated offspring that solicit extensively for food (Beecher et al. 1981b; Proffitt and McLean 1990; Hauber 2002). In addition, enhanced calling, without parental food rewards, needlessly increases the risk of attracting predators (Haskell 1994; Briskie et al. 1999). Such recognition errors are expected to be common in communication systems with low signal variation and whenever the identity signal is close to the modal, i.e. the most common signature. Selection for accurate parent-offspring recognition should favour individuals or parents with distinct signals, since these individuals will be more easily recognized and less likely to be confused with others (Dale et al. 2001).

Here we use a captive population of Australian zebra finch (*Taeniopygia guttata*) which is an opportunistic breeder with biparental care (Zann 1996) to study individual recognition in parent-offspring communication after fledging. Zebra finches are ideal study organisms since they breed in loose colonies (Zann 1996) and offspring are fed during an extended post-fledging phase by their parents (Zann 1996). Recognition systems are expected to evolve under such breeding conditions, where offspring could potentially confuse their own mother and father with other food-provisioning parents, and we predict the spread of individual signatures in distance calls. After fledging, offspring start to elicit this specific distance call that is used in the context of parent-offspring communication and is replaced by the begging call once the parents have approached (also called ‘long tonal call’ in Zann (1996)). This distance call is not only used in the context of food-provisioning but also whenever fledglings try to re-unite with their parents. In addition, individual signatures in distance calls may be important during social interactions within a flock and for mate recognition at the adult stage (Vignal et al. 2008).

Such a communication system allows testing critical hypotheses of parent-offspring recognition and identity signalling. Specifically we test whether offspring can discriminate between distance calls of their own parents (mother and father) and unrelated adults. We predict that fledglings are able to recognize their parents and will respond stronger to their parents than to non-parents. A stronger response can be manifested through an increase in number of response calls, shorter latency to respond and/or changes in call characteristics related to call urgency or motivational status (Morton 1977). In addition, we test whether an offspring's response to unrelated individuals is related to the gender of adult calls. It is known that male distance calls are learned and show high variability while female calls are innate and show relatively low variation (Zann 1996; Forstmeier et al. 2009). In the light of these sex-specific developmental trajectories of adult calls, we predict that offspring will commit more recognition errors in relation to female calls and will therefore respond more often to unrelated female than unrelated male calls. Alternatively, the response pattern to unrelated adults may reflect a chick's strategy due to behavioural differences, namely aggressiveness or discriminatory abilities in feeding behaviour, of males and females. Here, we collect data about adult call variability and adult behaviour to assess whether a fledgling's response pattern to unrelated adults reflects more likely recognition errors or a feeding strategy.

METHODS

Subjects and housing

Fledgling zebra finches (*Taeniopygia guttata*) used in the present study were from a captive population held at the Max Planck Institute for Ornithology in Seewiesen, Germany (imported from Sheffield University/UK). All fledglings tested in this study are descendants from birds breeding in one of six aviaries each of which held six breeding pairs. The sex of the offspring was determined using molecular methods (Griffiths et al. 1998). Temperature in the rooms was maintained at 24±1°C and relative humidity ranging from 40 to 60%. Rooms were illuminated by full-spectrum fluorescent light (Osram Lumilux T5 FH 28W/860 Daylight) and the light:dark period was 14:10 h. All birds received a millet seed mixture, cuttlefish, grit, water ad libitum on a daily basis and a multivitamin supplement once per week. All recognition trials were conducted between November 2007 and February 2008. Nestlings were weighed and colour-banded with a brood-specific plastic colour ring at 8 days of age. Aviaries were checked twice a day for newly fledged birds.

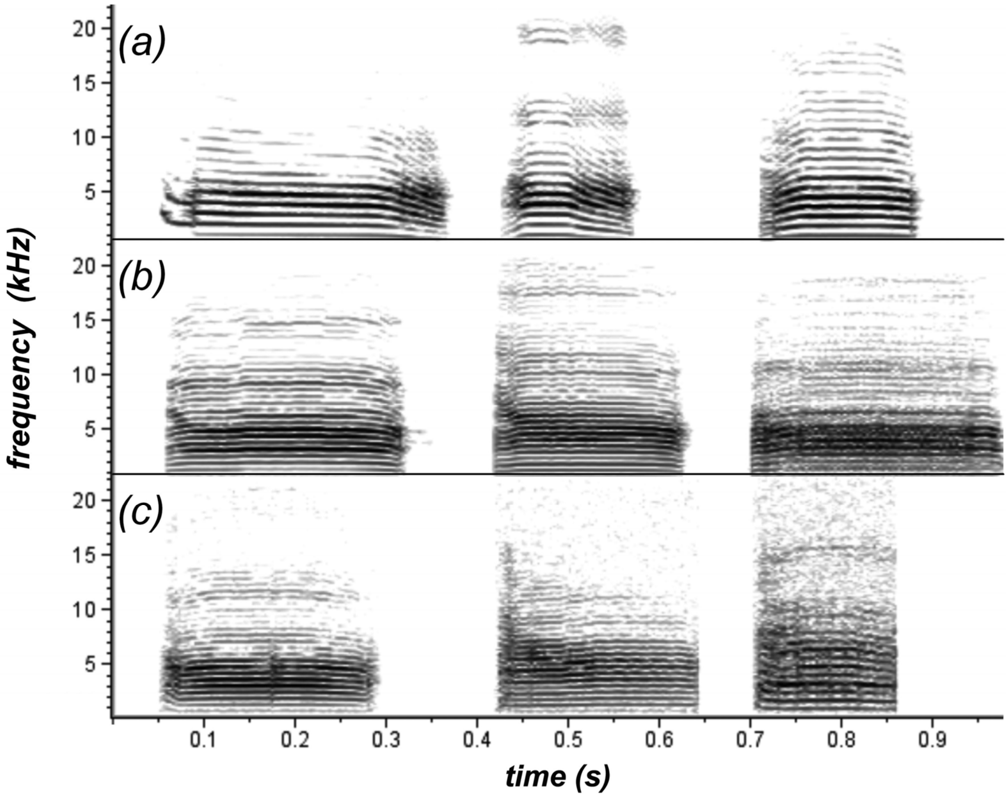

Figure 3.1
Sonograms showing inter-individual variation in distance calls of three different males (a), females (b), and fledglings (c).

Playback protocol

The playback experiment was intended to simulate a situation where a fledgling lost visual and acoustic contact to its parents. In this context, fledglings are expected to react to distance calls of their parents (Zann 1996). Recently fledged chicks (4.59 ± 2.01 days after fledging, range 1-9 days) were transferred from their natal aviary to a sound-attenuated chamber (70cm x 50cm and 50cm) equipped with a small metal wire cage containing a single perch, a microphone (C2, Behringer GmbH, Willich) and a small loudspeaker (I-Trigue, Creative Ltd., Dublin). We recorded (sampling rate: 44 kHz, amplitude resolution: 16 bit) the fledglings' response calls with a microphone that was connected to a solid state recorder (Microtrack II, M-Audio, Irwindale). Fledglings were allowed a 2 minutes 'acclimation phase' in the new experimental cage, before the recognition trials started. Preliminary tests had shown that fledglings calm down shortly after transferring them to the sound-attenuated chamber and the relatively short 'acclimation period' of 2 minutes proved to be long enough for testing a fledgling's response to adult calls.

In a playback experiment, we investigated a fledgling's acoustic response to its social parent's calls and to calls of unrelated adults (for examples of distance calls see figure 3.1). The stimulus calls of the genetically unrelated female and male (mean relatedness between chicks and unrelated adults: r=0.019, max r=0.125. Relatedness was measured based on a pedigree of 5 generations and calculated with the software Pedigree Viewer 5.1.) were calls of randomly chosen adults from this study population that lived in a different, acoustically separated room than the fledglings. For each fledgling within a brood we played back different unrelated adult calls. To find the most representative call recording for each individual we proceeded as follows (see Forstmeier et al. 2009 for details). We extracted from each call recording of every bird recorded in our population (N=13,815 calls of 806 individuals) 33 call parameters that are routinely calculated by SAP version 2.063 (Tchernichovski and Mitra 2004) as well as the first 12 mel-frequency cepstral coefficients calculated by Voicebox (Speech Processing Toolbox for MATLAB, written by M.Brookes, Imperial College, UK; http://www.ee.ic.ac.uk/hp/staff/dmb/voicebox/voicebox.html). These 45 call parameters were reduced to 12 principal components with Eigenvalues larger than one, and we calculated for every individual the centroid in this 12-dimensional space across its repeated recordings. We then took the euclidian distances of each call from the individual's centroid, and selected the recording that showed the smallest deviation. The same adult stimulus call was repeatedly used for all playback trials with the same fledging subject. Using a single call per individual will lead to inflated variance components of the random terms (i.e. individual and brood) but will lead to correct test statistics of the fixed effects, hence our analyses do not face the problem of pseudoreplication. Each fledgling was only tested once and presented with four series of different stimulus calls: mother, father, unrelated female, unrelated male. Each series lasted 30 seconds and contained a first part of 10 seconds where the stimulus call was repeatedly played back 3 times at intervals of 5 seconds (i.e., at 0, 5, and 10 sec from the start of the series). The playback phase was followed by a silent period of 20 seconds and followed by a new series with another stimulus call. The 4 series were broadcasted at random and repeated once, i.e. we investigated a fledgling's acoustic response to six stimulus calls of its father, mother and unrelated male and female. We tested for order of presented stimuli effects on the absolute and relative response to both parents and to calls of unrelated adults. Order effects never explained a significant part of the variation and were not included in the final models.

Fledgling call rate

We quantified the acoustic response of fledglings (83 individuals from 30 broods) to stimulus calls by measuring (1) the number of calls within 5 seconds following the stimulus calls and (2) the latency (ms), measured as the time between the end of the stimulus call and the chick's first response call. 6 out of 83 chicks never responded to any stimulus call and were excluded from further analyses. If a chick did not respond to a stimulus call, we scored number of response calls as 0, while latency was not scored. Accordingly, sample sizes and therefore also denominator degrees of freedom in the analyses differ when analyzing treatment effects on call number and call latency. In cases where the chick's response call overlapped the stimulus call, latency was scored as zero. Latency and the number of calls are highly correlated ($r=-0.451$, $p<0.001$, $N=209$), i.e. the more a chick responded to a stimulus, the shorter the latency. For analysis, both parameters were combined by calculating the first PC of a principal component analysis. Principal component 1 explained 71.85% of the variation.

Fledgling call characteristics

In addition, we quantified acoustic parameters of the fledglings' response calls using Sound Analysis Pro (SAP) software (Tchernichovski and Mitra 2004). SAP is specifically developed for the analysis of spectral-based features of zebra finch vocalizations. Compared to other methods that use visually assessed measurements from spectrograms, SAP uses complex algorithms to calculate values for each millisecond of the call and provides means and variances of those values (Tchernichovski and Mitra 2004). For all our analysis we used the default settings of SAP. Response calls were selected and analysed using the batch module of Sound Analysis Pro. For analysis, we used the response call with the best signal to noise ratio (i.e. the loudest call) elicited after each stimulus call. We refrained from analyzing all acoustic parameters and focused on acoustic parameters that are suggested to relate to motivational status and call urgency in birds (Morton 1977). These parameters were mean amplitude (dB), amplitude modulation, call length (ms) (Ficken 1990; Leavesley and Magrath 2005), mean frequency (Hz) (Ficken 1990; Leavesley and Magrath 2005) and frequency modulation. Frequency modulation is estimated based on time and frequency derivatives across frequencies. If the frequency derivatives are much higher than the time derivatives, frequency modulation is low and vice versa (for details see Tchernichovski and Mitra 2004). Amplitude modulation is the overall time-derivative power across all frequencies within a range (for details see Tchernichovski and Mitra 2004). Mean frequency provides a smooth estimate of the centre of derivative power. In contrast to peak frequency, mean-frequency does not 'stick' to any frequency trace (for details see Tchernichovski and Mitra 2004).

Adult behaviour in parent-offspring interactions

Observations of adult behaviour were carried out throughout the breeding season (50 sessions of 5 min for each of the 6 aviaries, i.e. 25 h in total). We recorded (1) whether adult birds fed their own or unrelated offspring and (2) aggression of adult birds towards fledglings. In total we recorded 56 feeding events by 15 different males and 49 events by 17 different females. Adult attacks on fledglings were observed 330 times involving 34 out of the 36 males and 32 out of the 36 females.

Statistical analyses

General statistical methods

All statistical analyses were performed with R 2.8.0 (R Development Core Team 2008). The standard model diagnostics of non-normal errors, non-constant error variance and the presence of outliers were performed on each of the final models according to Fox (2002). In all mixed model analyses, we used a stepwise backward procedure and tested the significance of predictor variables in a hierarchical fashion, retaining lower order effects in the analyses in case a higher order term was significant.

Fledgling call rate and call characteristics

To analyze the effects of stimulus calls on a fledgling's quantitative (number of response calls, time to first response call) and qualitative (call parameters) response, we used linear mixed-effects models (package nlme, Pinheiro et al. 2006) with the sex of the adult (2 levels; i.e. male, female), relatedness (2 levels, i.e. parents, unrelated) and the sex of the offspring (2 levels; i.e. male, female) as fixed factors, and individual nested within brood (i.e. social pair, 30 levels) as a random factor. Age after fledging was initially included as a covariate in all analyses. As it never explained a significant part of the variation it was excluded from the final models. The denominator degrees of freedom of the test statistics of mixed models are computed according to Pinhero and Bates (2000, page 91).

We tested whether fledglings responded to both parents with the same number of response calls or showed a preference towards one parent. For this analysis we run separate chi-square tests for each chick, comparing the number of response calls towards mother and father. Fisher's combined probability was used to combine probabilities of chi-square tests of each chick.

Sex differences in call variability

To test for sex-differences in adult distance calls and to quantify individual differences in distance calls for both adult females and males, we performed three separate discriminant function analyses (DFAs) (package MASS, Venables and Ripley 2002). All DFAs were done using 10 acoustic parameters (call duration, mean pitch, mean principal contour, mean frequency modulation, mean entropy, mean pitch goodness, mean frequency, variance in frequency modulation, variance in entropy and variance in amplitude modulation). Parameters were BoxCox transformed prior to the DFA to approach a normal distribution. For the DFA on sex differences we used a randomly selected single call from 30 adult females and 30 adult males. For the DFAs on intra-sex differences we used 10 calls per individual and a total of 27 individuals per sex. The calls were randomly selected for each individual. For all DFAs a cross-validated (leave-one-out) procedure was used to fit a left out call into the multidimensional signal space calculated from all calls but the one left out. The correct assignment rate was calculated as the percentage of calls assigned to the correct sex or individual respectively

RESULTS

Vocal recognition in parent-offspring communication

From a total of 83 tested fledglings, 6 chicks did not respond to any adult call. Fifty-nine fledglings responded to both the calls of parents and unrelated adults. Fourteen fledglings reacted to their parents only, while 4 individuals responded to unrelated adult calls only and not to their parents calls. In a first model we analyzed the effect of relatedness on a fledgling's response. Fledglings reacted faster (call latency; relatedness: $F_{1,153.3}=34.48$, $p<0.001$) and with more calls (number of calls: relatedness: $F_{1,245}=31.40$, $p<0.001$) towards distance calls emitted by their parents than to distance calls of unrelated adult calls (Figure 3.2). In a second step we analyzed a fledgling's response in relation to the gender and relatedness of adult calls. The latency of a chick's first response call depended on the relatedness but additionally on the sex of the adult stimulus call (sex x relatedness: $F_{1,154}=5.98$, $p=0.017$, figure 3.2a). In a post-hoc approach, we analyzed the reaction towards parents and unrelated adults separately. The reaction towards father and mother did not differ ($F_{1,61.8}=1.07$, $p=0.31$, figure 3.2a), whereas fledglings reacted faster towards unrelated females versus unrelated males ($F_{1,48.1}=6.83$, $p=0.01$, figure 3.2a). A similar response pattern was found in relation to number of response calls. The reaction towards father and mother did not differ ($F_{1,82}=0.19$, $p=0.67$, figure 3.2b), whereas fledglings reacted faster towards unrelated females versus unrelated males ($F_{1,82}=5.63$, $p=0.02$, figure 3.2b). The interaction between sex and relatedness for call number was not significant (sex x relatedness: $F_{1,246}=2.73$, $p=0.10$, figure 3.2b). Combining both measures using a principal component analysis we found that the reaction towards adult calls depended on the relatedness but additionally on the sex of the adult (PC1; sex x relatedness: $F_{1,152}$=4.57, p=0.034). The sex of the offspring had no effect on call latency ($F_{1,62.3}$=1.90, p=0.17) and the number of response calls ($F_{1,79.70}$=0.10, p=0.76) or PC1 ($F_{1,65.80}$=0.41, p=0.52).

The response pattern of fledglings towards calls of unrelated adults might be related to the frequency of male versus female feedings of unrelated offspring or to male versus female aggressiveness towards fledglings. On average, males were feeding fledglings that were not their social offspring in 41% of their feedings (N=56 feeding observations on 15 males), and females fed non-social offspring in 65% of their feedings (N=49 observations on 17 females). Male and female feeding behaviour did not differ significantly (glm with quasibinomial error distribution; t_{30}=-1.48, p=0.15). Throughout all of our observation sessions, the average male was seen to attack a fledgling 5.2 times, while the average female attacked fledglings 4.0 times. Again, aggressiveness towards fledglings was not significantly different between males and females (t-test on sqrt-transformed data; t_{70}=1.28, p=0.21).

Call characteristics of fledglings differed in their response to parents and unrelated adults. For the comparison of call characteristics we only used individuals that responded to their parents and to the same sex of unrelated adults. These were 40 fledglings that reacted to both female calls and 29 individuals that reacted to both male calls. Individuals that responded to both female calls, responded louder, at a higher mean frequency and the calls were longer towards their mother while there were no effects on frequency modulation and amplitude modulation (Table 3.1). The same pattern was found in individuals that responded to both male calls. Calls emitted towards their father were louder, at a higher mean frequency and the calls were longer while there were no effects on frequency modulation and amplitude modulation (Table 3.1). Overall, the calls emitted towards parents were of a higher urgency compared to response calls towards unrelated adults. The sex of the offspring had no effect on any of the measured call characteristics (all p>0.12)

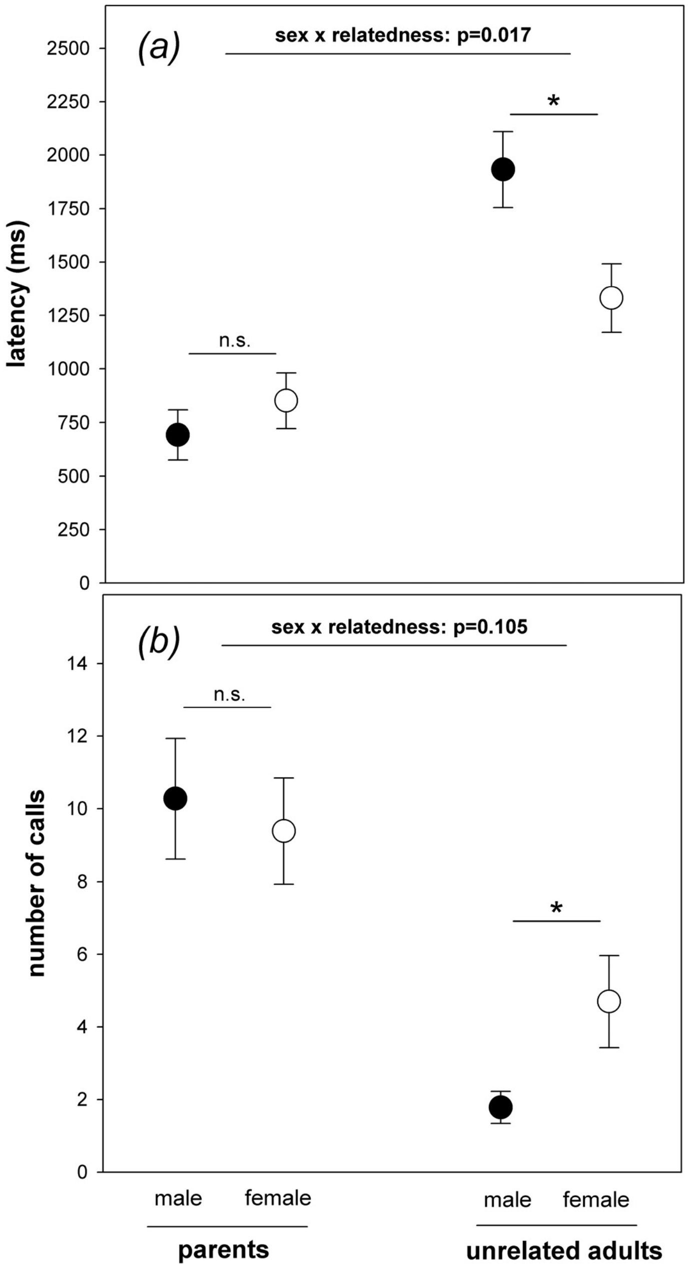

Figure 3.2

Fledglings' response (a) call latency, (b) number of response calls, in relation to distance calls of their parents and unrelated adults. Shown are means ± s.e.. Significance is indicated with asterisks, where one asterisk means $p<0.05$.

Individual recognition in parent-offspring communication

Figure 3.3 illustrates the proportion of response calls each nestling emitted towards its mother and father. Thirteen (9 male, 4 female) fledglings reacted towards the mother only, whereas 13 (8 males, 5 females) fledglings responded only towards the father. The sex of the offspring did not affect the relative reaction towards both parents (Mann-Whitney U Test: $U=562.5$, $p=0.36$). Visual inspection of figure 3.4 indicates a 'complex' distribution with two distinct peaks around 0 and 1. Fledglings either reacted to their father (i.e. values close to 0), their mother (i.e. values close to 1) or to both parents (i.e. values between 0 and 1). The response pattern clearly deviates from a normal, unimodal distribution (Fisher's Combined Probability, $722.22>176$, $p<0.001$; $\chi_{0.001,122}^2 = 176$) and fledglings that responded to a single parent only (13 towards mother, 13 towards father) are highly overrepresented. These findings indicate that at least some fledglings use individual signatures, eventually in combination with signatures that code for the sex of an adult bird, to recognize their parents.

Response to unrelated adults

The response to unrelated females depended on a fledgling's relative response towards its parents. Fledglings that showed a preference for their mother also reacted more towards unrelated females than unrelated males. Out of 27 fledglings that showed a preference for their mother, 24 responded significantly more towards an unrelated female (binomial test: $p<0.001$). In contrast, a preference for their father had no effect on reaction towards unrelated males and females. Out of 21 fledglings that reacted stronger towards their father, only 12 responded more to unrelated males (binomial test: $p=0.66$).

The response to unrelated adults depended on whether fledglings responded to one or both parents. Out of those 26 individuals that responded to a single parent only, 12 also reacted towards unrelated parents. In contrast, 42 out of 47 fledglings that reacted to both parents also reacted to unrelated adults. Individuals that reacted to both parents were more likely to respond to unrelated adults compared to fledglings that reacted to a single parent only (Fisher's Exact Test: $p<0.001$, odds ratio=9.42). Body mass at day 8, as an indicator of past growing conditions, did not predict whether fledglings responded to one or both parents (t test: $t_{1,70}$=0.10, p=0.92).

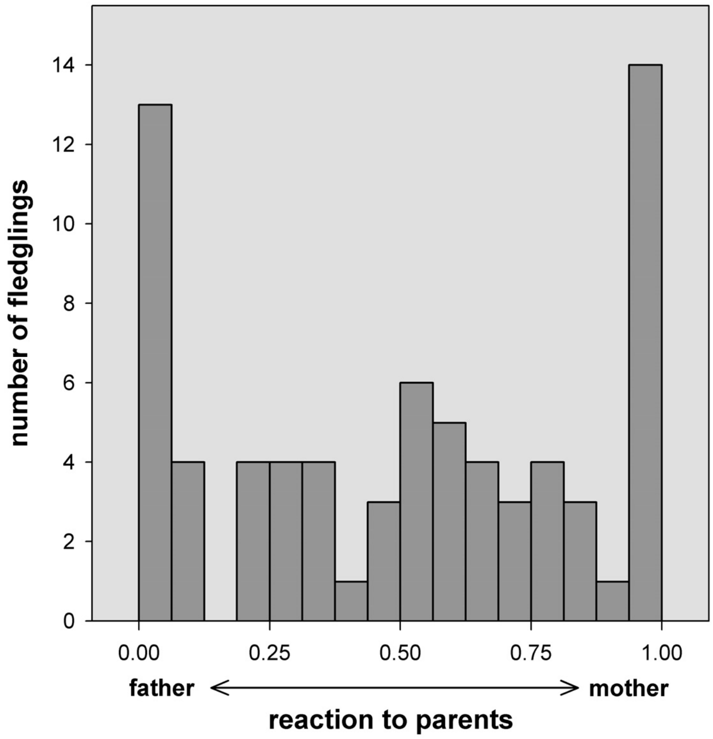

Figure 3.3
Frequency distribution of fledglings' response pattern towards their mother and father. Illustrated is the relative response in proportions, where values of 0 and 1 refer to fledglings that reacted to a single parent only. Note that 1 bin covers 6.25 %, e.g. the last bin covers the range from 93.75% until 100%.

Recognition errors - call variability of female and male calls

A discriminant function analysis (DFA) using a single call of 60 individuals (30 individuals/sex) reveals that 54 calls (26 male calls, 28 female calls, overall 90%) got assigned to the right sex. The calls of both sexes are highly distinctive and can be discriminated better than by an expected 50% correct assignment by chance (binomial test: p<0.001).

In a second step we investigated sex-specific variation in distance calls. 72% of the female calls (194 out of 270) were assigned to the correct individual while male calls showed a 95% (257 out of 270) correct assignment rate (Fig. 4a, b). In both sexes, calls are individually distinct, but the assignment probability for male calls is significantly higher than for female calls (t test: t_{52}=4.03, p<0.001).

DISCUSSION

Our study demonstrates that zebra finch fledglings recognized their parents and that many fledglings responded to distance calls of unrelated adults. The significant interaction term between sex and relatedness on latency and our combined measure of response latency and the number of response calls (i.e. PC1) demonstrates that offspring reacted more towards unrelated females than unrelated males. This sex-specific response to unrelated adults may reflect a strategy of fledglings but may additionally be related to differences in signal variation among sexes. Female calls show low intra-sexual variation and are more similar to each other than male calls. We further discuss whether the higher response rate to unrelated females may be attributed to recognition errors or a strategic investment of fledgling birds.

Individual recognition versus class-recognition

Fledglings reacted faster and emitted more response calls towards their parents than to unrelated adults. In addition, the response to their parents differed among fledglings, where around a third of the individuals reacted only towards one individual parent, irrespective of the parent's sex. This response pattern demonstrates that at least some fledglings are able to discriminate among two equally familiar adult calls and use individual signatures, in contrast to class-signatures (e.g. relatedness), to recognize their mother and father. These individual signatures are most likely learnt and may be composed of components that encode the sex in combination with components encoding the individual. Such a fine discrimination of parental calls can be facilitated by the mere exposure or familiarity to the stimuli or alternatively result from differential reinforcement at a similar exposure of the different stimuli. A preference towards one of the parents may reflect the history of previous parent-offspring interactions among individuals. In several species, individual adults feed a subset of the brood, rather than each adult feeding all young (Leedman and Magrath 2003;

Table3.3 Means±SE, estimates, and test statistics of response calls of fledglings towards distance calls of parents (mother, father) and unrelated adult (female, male) calls

	Mother	Father	Female	Male	Comparison	Estimates	SE	df	t	p
Amplitude (dB)	32.11±1.00	29.48±1.34	29.96±0.93	26.08±1.31	Mother vs. female	2.15	0.92	39	2.34	0.025
					Father vs. male	3.00	1.06	28.36	2.82	0.009
Call length (ms)	215.80±11.67	201.39±168.70	190.43±9.88	168.70±10.67	Mother vs. female	25.38	10.32	39	2.46	0.019
					Father vs. male	31.11	13.90	28.77	2.24	0.033
Mean frequency (Hz)	3991.85±81.53	3819.32±101.49	3764.68±84.37	3553.20±89.14	Mother vs. female	218.20	84.09	38.08	2.60	0.013
					Father vs. male	238.80	98.65	28.66	2.42	0.022
Frequency modulation	16.23±1.37	15.84±1.68	16.37±1.34	15.02±1.25	Mother vs. female	0.19	1.45	33.61	0.13	0.90
					Father vs. male	0.63	1.64	28.87	0.38	0.70
Amplitude modulation (1/ms)	0.0092±0.0005	0.0092±0.0007	0.0093±0.0005	0.0079±0.0006	Mother vs. female	0.0004	0.0006	34.42	0.07	0.94
					Father vs. male	0.001	0.0007	28.59	1.67	0.11

Note that frequency modulation does not have a unit

Draganoiu et al. 2006). A recent study in black redstarts (*Phoenicurus ochruros*), a species that shows 'brood division' after fledging, demonstrates that individual parents only responded to the subset of distance calls of fledglings they were feeding (Draganoiu et al. 2006). In line with these results, the observed response pattern of young zebra finches may reflect brood division where a fledgling only or mainly responded to the parent that was feeding it. Alternatively, a stronger reaction towards one parent may reflect the absolute amount of investment in offspring feeding of the mother or the father. Both scenarios are possible, not mutually exclusive, and more detailed behavioral observations of parent-offspring interactions after fledging are needed to distinguish among both hypotheses.

Not only did fledglings react more to their parents, but also they altered call characteristics. It is widely known that the motivational status of a signaller affects call structure (Morton 1977). In situations of high urgency, bird and mammal species have been shown to produce longer calls and vocalisations of higher frequency (Ficken 1990; Leavesley and Magrath 2005; Furrer and Manser 2009). Here, we propose that changes in these call parameters in relation to an increasing urgency likely reflect the consequence of selection for call loudness. The amplitude of calls is expected to be a main call character under selection because of its direct effect on detection probability (Brumm and Slabbekoorn 2005). Call amplitude is mainly determined by the air pressure in a bird's air sacs, which affects the airflow through the syringeal systems (Plummer and Goller 2008). By increasing the airflow through the syrinx, a bird will not only change vocal amplitude but calls will additionally be altered in length and frequency (Plummer and Goller 2008). These accompanied changes highlight that the expression of each call parameter within a complex multi-component signal can be limited by selection on other call components. How these constraints on signal production affect the perception of single call component is unknown and receivers may use the accompanied changes in frequency or call length and not the trait under selection, i.e. amplitude to assess the urgency of a call.

Responses to unrelated adults in parent-offspring communication

Many fledglings responded to unrelated adult calls. Most importantly, the reaction was more common towards unrelated females than males. Our findings are in line with previous studies that have shown a stronger vocal response (Vicario et al. 2001) and neuronal activation (Gobes et al. 2009) of adult birds towards female distance calls. The stronger response towards unrelated females is most likely not due to behavioural differences between the sexes. Males and females did not differ significantly in the likelihood to allocate food to unrelated fledglings or in the aggressiveness towards fledglings. The observed percentage of adult feedings to unrelated fledglings was surprisingly high and unexpected. The occurrence of extra-pair paternity and egg dumping did not explain these observed cases (unpublished data). Therefore, these results may rather be the consequence of the housing situation, which may not mimic the natural conditions perfectly. In our aviaries, parent-offspring interactions may be complicated through limited space where family units (i.e., food-provisioning adult and its offspring) cannot easily escape from unrelated food-soliciting fledglings.

The stronger reaction towards female calls can also be discussed in the light of recognition errors. Female calls show lower intra-sexual variation, and individual female voices are more difficult to discriminate than male voices, at least in a statistical approach using discriminant function analyses. In our study, female calls were assigned to the correct individual in about 72% of the cases, while male calls were assigned to the correct individual in almost all cases (95%). An assignment rate of 72% is still high and demonstrates that female calls possess individually distinctive features (see also Vignal et al. 2004). However, it also shows that a substantial proportion of female calls (28%) were statistically assigned to the wrong female which could account for the relatively high number of responses to unfamiliar female calls in our study. The proximate reason for intersexual differences in variation in distance calls might be due to different selection pressures acting on male and female voices. Male calls are learnt and show high intra-sexual variation (Forstmeier et al. 2009). Learning processes are often not perfectly precise thereby adding variance to the learnt trait and facilitating the accurate recognition of the father's individual signature. In contrast, distance calls of adult females are innate (Forstmeier et al. 2009), show low intra-sexual variation and the mother's call is more likely to be confused with another female call.

Another result supports the idea that recognition errors are mainly due to low between-individual variation in female calls. Fledglings, which showed a preference for their mother, also reacted stronger to unrelated females. This relationship was only observed in reactions to the mother, i.e. fledglings that reacted more to their father did not react more strongly to unrelated males. This sex-specific response rate indicates that fledglings will commit recognition errors in relation to female calls as long as they respond to their mother. In cases where fledglings responded to a single parent only, they responded significantly less to unrelated adult calls than fledglings that responded to both parents. Fledglings that responded to both parents show an environmentally induced or genetic predisposition to be less choosy. Individuals in states of high need may adopt more risk-prone strategies, trying to solicit food from unrelated individuals or the existence of highly consistent heritable individual variation in behavioural strategies, also referred to as personalities, may account for the differences in responsiveness towards adult calls among fledglings (Drent et al. 2003).

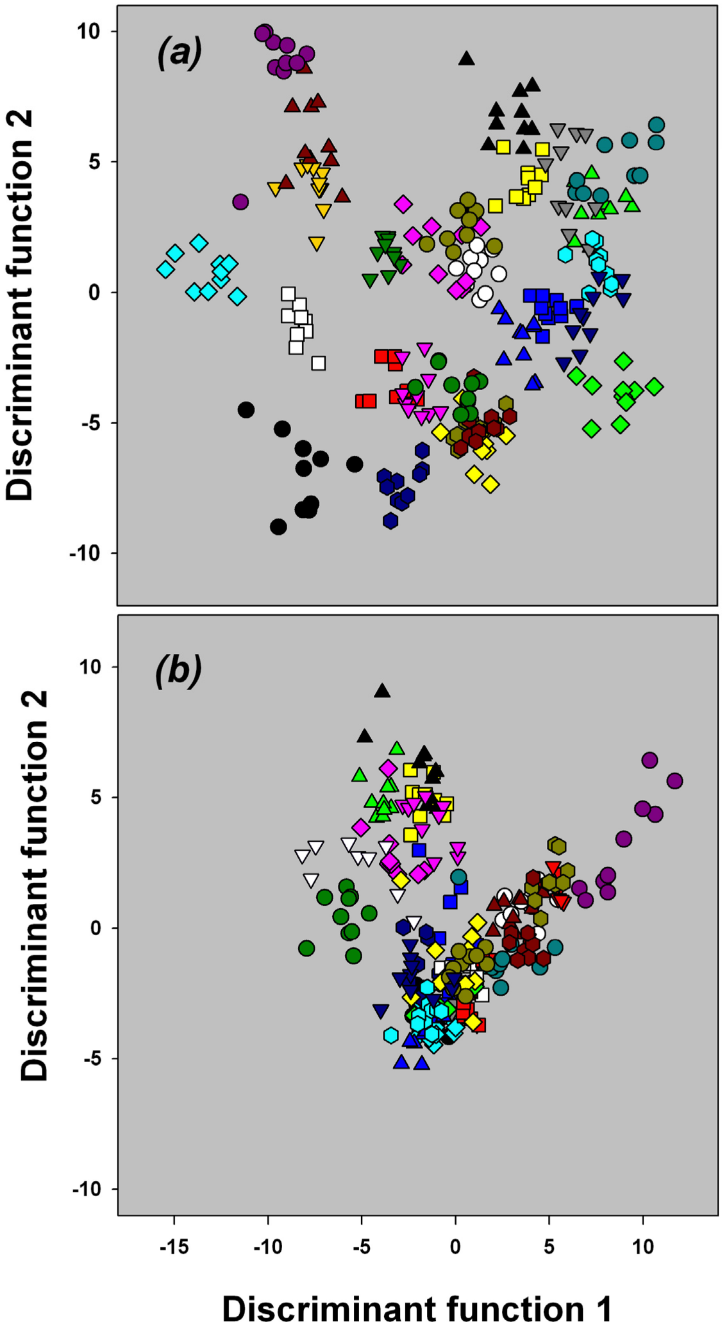

Figure 3.4

Discriminant analyses based on 27 males (a) and females (b). Plotted are the two first discriminant functions that explain 68.8% (DF1: 42.7%, DF2: 26.1%) of the variation in males and 67.7% (DF1: 36.0%, DF2: 31.7%) of the variation in females. Note that the DFA's produced 10 discriminant functions and the figure therefore underestimates the differences in call signatures between individuals.

This study highlights the importance of responses to unrelated adults in understanding parent-offspring communication specifically and recognition processes in general. While many studies have demonstrated that offspring recognize their parents it remains an open question how signal properties facilitate or complicate recognition processes. To conclusively demonstrate that calling towards unrelated adults reflects recognition errors and not a strategy of fledglings we still need careful experiments linking a fledgling's responsiveness with the similarity in adult signal properties important in individual recognition. Such studies will yield insight into costs and benefits of individual signatures and test fundamental assumptions of the spread of individual signatures via negatively frequency-dependent selection.

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Chapter 4

Do zebra finch parents fail to recognize their own offspring?

Hendrik Reers, Alain Jacot, Wolfgang Forstmeier

Abstract

Individual recognition systems require the sender to be individually distinctive and the receiver to be able to perceive differences between individuals and react accordingly. Many studies have demonstrated that acoustic signals of almost any species contain individualized information. However, fewer studies have tested experimentally if those signals are used for individual recognition by potential receivers. While laboratory studies using zebra finches have shown that fledglings recognize their parents by their "distance call", mutual recognition using the same call type has not been demonstrated yet. In a laboratory study with zebra finches, we first quantified between-individual acoustic variation in distance calls of fledglings. In a second step, we tested recognition of fledgling calls by parents using playback experiments. Using a discriminant function analysis, we show that individuals are highly distinctive and most measured parameters show very high potential to encode for individuality. Zebra finch parents do react to calls of fledglings, however they do not distinguish between own and unfamiliar offspring, despite individual distinctiveness. Our results demonstrate the importance of adopting a receiver's perspective and suggest that variation in fledgling contact calls might not be used in individual recognition of offspring.

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Whenever information is transferred between two individuals, this happens via a signalling system. Signalling systems consist of three parts, the sender, the signal and the receiver. Is the signal used to indicate the identity of the sender to the receiver, for example a young fur seal (*Arctocephalus tropicalis*) calling for its mother in a nursing colony (Charrier et al. 2001a), certain signal properties are needed to ensure reliable recognition between sender and receiver. The signal must provide between-individual variation, combined with within-individual uniformity, to function as an individual signature (Halpin 1991; Tibbetts and Dale 2007). It is well established that between-individual variation in acoustic signals commonly occurs. An extensive body of literature has shown that virtually all acoustic signals of animals across many taxa show between-individual variation (e.g. Jouventin et al. 1999; Charrier et al. 2001a; Sharp and Hatchwell 2005). From a receiver's perspective, an animal must be able to perceive these between-individual differences in order to respond accordingly (Johnstone 1997).

Acoustic individual recognition is essential in a variety of contexts with repeated social interactions, of which parent-offspring communication received a lot of attention in recent years (e.g. Aubin and Jouventin 1998; Charrier et al. 2001a; Draganoiu et al. 2006). The ability to discriminate calls of single individuals from other conspecifics is especially important in a colony with hundreds or thousands of individuals communicating simultaneously (e.g. Beecher 1988; Aubin and Jouventin 2002). Parents and their mobile chicks often use calls to reunite after parents have left their offspring alone (e.g. Stevenson et al. 1970; Charrier et al. 2001b). Playback experiments have shown that parents and mates perceive individual acoustic differences between individuals and are able to recognize the sender acoustically (e.g. Barg and Mumme 1994; Draganoiu et al. 2006). Especially acoustic properties that relate to the time-frequency pattern of a sound (e.g. frequency modulation, frequency range or duration) have been shown to be important for individual distinctiveness and individual recognition (e.g. Aubin and Jouventin 2002; Vignal et al. 2004; Vignal et al. 2008).

Zebra finches (*Taeniopygia guttata*), which are opportunistic breeders with biparental care, nest in loose colonies of up to dozens of pairs, and offspring are fed during an extended post-fledging phase by their parents (Zann 1996). Recognition systems are expected to evolve under such breeding conditions, where parents face the risk of potentially confusing their own offspring with other fledglings. The situation, in which recognition of fledglings by its parents is expected to be most important, is after separation when parents and fledglings need to reunite (e.g. after parental feeding trips or predator disturbance). Observations in the wild indicate that fledglings and parents use "distance calls" to reunite (Zann 1996). This ability is expected to be very important since the location of a young is a very unreliable indicator of its identity. Especially so in colonially breeding species in which young often change their location and where young are likely to intermingle (e.g. Charrier et al. 2001a; Aubin and Jouventin 2002; Knörnschild and von Helversen 2008; Mulard et al. 2008). Unfortunately, detailed information about the role of parents and their fledglings in the reuniting and recognition process are still missing in the scientific literature. In captivity, it has been shown that young zebra finches vocally respond using distance calls to specifically react to distance calls of their parents and mostly ignore calls of other individuals when separated from their parents (Jacot et al. 2010). However, young occasionally also respond to distance calls of non-parents (Zann 1996; Jacot et al. 2010). In order to avoid feeding unrelated young, parents are expected to be able to recognize their own offspring.

Interestingly however, Jacot et al. (Jacot et al. 2010) reported that in an aviary situation, about 50% of feedings are directed to foreign offspring. This observation is puzzling considering that Levrero et al (Levréro et al. 2009) have shown in their study that captive zebra finch parents recognize the begging calls of nestlings one day before fledging. Begging calls are still used once fledged, but only to obtain food in short distance communication, not to reunite after separation (Zann 1996; Jacot et al. 2010). We therefore chose distance calls of fledglings to investigate acoustic individuality and its use in parent-offspring recognition in our captive population. It has been shown that distance calls of adults show individual signatures and are used in mate recognition in which both sexes are able to recognise their partners (Vignal et al. 2004; Vignal et al. 2008). However, the ability of parents to use the early distance calls of fledglings to recognise their offspring is unknown and has not yet been experimentally tested. We expect parents to recognize their own fledglings' distance calls for two reasons. First, to make reuniting efficient given the risk that offspring intermingles with conspecific young and second, to enable parents to invest in their offspring and to avoid feeding of unrelated chicks. In a first step we quantify individual and brood signatures in fledgling distance calls statistically. In a further playback experiment we then test acoustic parental recognition of fledglings with distance calls of their own versus unfamiliar (alien) fledglings.

METHODS

Ethical note

The study was approved by the animal care and ethics representative of the Max Planck Institute for Ornithology.

Study subjects

Fledgling zebra finches used in the present study originated from a captive population held at the Max Planck Institute for Ornithology in Seewiesen, Germany. All fledglings and parents used in this study are from breeding pairs kept in aviaries that held six breeding pairs. The sex of the offspring was determined using molecular methods (Griffiths et al. 1998). Temperature in the rooms was maintained at 24±1°C and relative humidity ranging from 40 to 60%. Rooms were illuminated by full-spectrum fluorescent light (Osram Lumilux T5 FH 28W/860 Daylight) and the light:dark period was 14:10 h. All birds received a millet seed mixture, cuttlefish, grit, water ad libitum on a daily basis and a multivitamin supplement once per week. All recognition trials were conducted between May and August 2009. Aviaries were checked twice a day for newly fledged birds. Nestlings were individually marked by numbered alloy bands when eight days old.

We used distance calls of 84 fledglings recorded in a previous breeding season (2007/2008) to investigate individual distinctiveness using discriminant function analysis (DFA). These individuals were used as unfamiliar (alien) stimulus calls for the playback experiment testing parent-offspring recognition.

Calls from 64 fledglings from the actual breeding season 2009 were used as 'own' stimulus for their social parents in the playback experiment.

Distinctiveness in fledging distance calls

To investigate acoustic individuality, brood and sex differences in fledgling distance calls, we used 493 calls from 84 fledglings (40 females and 44 males; age: 22.5±2.1) originating from 30 broods from breeding season 2007/2008. For most of the fledglings, no stimulus calls were used during the recording of calls, however for about 10% of fledglings that did not call at all, we used parental calls to stimulate fledgling calling. Fledgling calls were analysed using Sound Analysis Pro software 2.065 (Tchernichovski and Mitra 2004), a computer program specifically developed for zebra finch vocalizations, using standard settings. We extracted the following acoustic features to characterize the acoustic variability within and between fledglings: 1) call duration (ms), 2) mean amplitude (dB), 3) variance in amplitude modulation, 4) mean frequency (Hz), 5) mean frequency modulation, 6) variance frequency modulation, 7) mean entropy, 8) variance in entropy, 9) mean pitch (Hz) and 10) mean pitch goodness. These parameters were chosen from a larger pool of parameters because of their usefulness in discriminating between individual zebra finches (Jacot et al. 2010).

Parent-offspring recognition experiment

We simulated a situation in which a parent lost visual and acoustic contact to its family and where it is expected to react to distance calls of its young. This experimental set-up has proven successful in previous studies of parent-offspring and mate communication in zebra finches (Forstmeier et al. 2009; Jacot et al. 2010). We tested 42 adults from 21 breeding pairs with calls of 64 recently fledged young (mean±SD, 3.0±2.0 fledglings per pair, range: 1-7). The fledglings left the nest at 18.1±1.5 (range: 15-22) days of age and were recorded at 24.5±1.0 (range: 24-28) days of age. The parents were tested 3.1±2.1 (range: 0-8) days after the fledglings were recorded. Both parents were tested with one call from one own fledgling against three calls from alien fledglings (i.e. 128 trials, 64 own fledglings x two parents), which were randomly picked from 84 alien fledglings from the previous breeding season 2007/2008 (N randomly chosen alien fledglings=70, number of times used in experiments=5.49±2.56). Parents were tested singly in a sound-attenuated recording box (70cm x 50cm and 50cm high) which was equipped with a small metal wire cage containing a single perch, a microphone (C2, Behringer GmbH, Willich, Germany) approximately 20cm from the perch and a small loudspeaker (V20, Logitech, Morges, Switzerland) next to the microphone. The microphone was connected to a pre amplifier (SM Pro Audio, Melbourne, Australia) from which we recorded directly through a M-Audio Delta 44 (AVID Technology GmbH, Hallbergmoos, Germany) sound card onto the hard drive of a computer at a sampling rate of 44 kHz and 16 bit amplitude resolution using Audacity 1.3.7 (D Mazzoni, Canada, http://audacity.sourceforge.net/). Audacity was used to play back stimulus calls and to record the parent's response simultaneously. Both playback experiments and recording of stimulus calls were done in the same recording box with the same set up.

The playback experiment started with 150 seconds of silence to allow for acclimation of the parent to the recording chamber. For each parent we build a playback that consisted of calls of three different, unfamiliar chicks and one own young. For each of these stimulus birds we build a 30 second 'individual-unit' that consisted of the same call starting at 0, 5 and 10 seconds, followed by 20 seconds silence (Figure 4.1). Four of these individual units, each from a different fledging, were combined into a 120 seconds 'repeat unit' that was repeated once and used for both parents in each breeding pair. The order of stimulus individuals (own versus alien) within a repeat unit was randomized. In cases when parents had more than three offspring, we tested no more than three of their fledglings on a given day but continued the experiment the next day. For multiple playbacks on one day, each fledgling's playback was separated by 60 seconds of silence before the playback of the next fledgling started.

The parent's acoustic response was measured as the number of calls within the 5 seconds from the start of each stimulus call. We also measured the latency to call as the time from the start of the stimulus call to the parent's first response call. In cases when parents did not respond to a stimulus, latency was not scored, but the number response call was zero. Previous studies on zebra finches have shown that the number of calls and the latency to respond are reliable behavioural measures for acoustic recognition in both, adults (Vignal et al. 2008; Levréro et al. 2009) and young (Jacot et al. 2010). When comparing locomotor activity response, i.e. adults approaching stimulus calls, with a vocal response, i.e. adults responding to stimulus calls, previous studies in adult zebra finches have shown that the vocal response is a better measure for individual recognition (Gobes et al. 2009; Levréro et al. 2009).

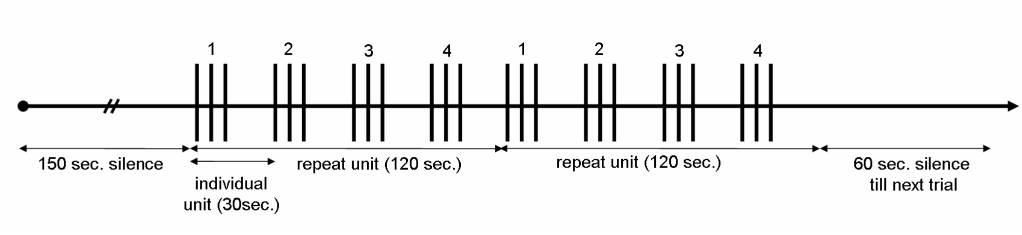

Figure 4.1
Playback design for fledgling stimulus. Each fledgling stimulus was used with both parents. The order of own and alien stimuli (1, 2, 3, 4) was randomized with the repeat unit.

Statistical analysis

General statistics

All statistical analyses were performed with R 2.10.0 (R Development Core Team 2010).

Acoustic individuality

To test whether individuals, broods and sexes can be distinguished statistically by acoustic parameters, we performed three discriminant function analyses (DFA). (1) We used 493 calls from 84 individuals (5.87±0.34 calls/individual) to test for individual distinctiveness. (2) We used averages for each acoustic variable from 43 individuals from 14 broods (i.e. genetic full siblings, no extra-pair or dumped siblings) with at least two recorded siblings to test for brood differences. (3) We used averages for each acoustic variable from 84 individuals (44 males, 40 females) of each acoustic variable to test acoustic differences between sexes. For the DFAs we used 8 call parameters. We excluded variance in frequency modulation because of high inter-correlation (i.e. r>0.8) with other parameters (variance in frequency modulation – variance in amplitude modulation, r=0.87; variance in frequency modulation – mean frequency modulation, r=0.85) (Seddon and Tobias 2010). In addition, mean amplitude was excluded because differences in amplitude can be due to differences in direction at which a fledgling is calling. The calls were assigned to individuals, broods or sexes using a cross-validated (leave-one-out method) DFA (package: MASS, Venables and Ripley 2002).

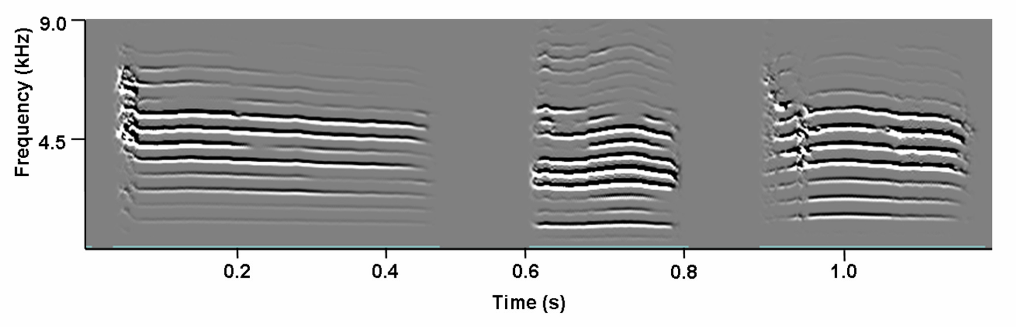

Figure 4.2
Three representative examples showing inter-individual variability of fledgling distance calls.

To describe the intra- to inter-individual variation ratio of each variable, we used the potential for individual coding (PIC) (Robisson et al. 1993) and we calculated repeatabilities based on linear mixed-effects models (Nakagawa and Schielzeth 2010). PIC is a measure of the ratio of inter-individual variation in comparison to intra-individual variation. For each variable we calculated the coefficient of variation (CV) as both, CVi (intra-individual CV) and CVb (inter-individual CV) according to the formula:

$$CV = 100 \times (SD / X) \times (1 + (1/(4 \times n)))$$

where SD is the sample standard deviation, X is the sample mean and n is the sample number (Robisson et al. 1993). PIC is the ratio of CVb divided by the mean of CVi of all individuals. PIC values above one are considered indicating potential for individual coding because the variation between individuals is larger than within individuals (Robisson et al. 1993). Repeatabilities were calculated based on linear mixed-effects models fitted by restricted maximum likelihood for all parameters using the rptR package (Nakagawa and Schielzeth 2010) for R 2.10.0 (R Development Core Team 2010). This was done to firstly get a second measure of intra- to inter-individual ratio of variation and secondly to compare PICs and repeatability measurements. Values for PICs and repeatabilities were compared in a linear regression. Prior to analysis all acoustic parameters were BoxCox-transformed to approach normality (package: car, Fox 2002).

Response to playback

To analyze the effects of stimulus calls on a parent's number of response calls, we used generalized linear mixed-effects models (glm) from the R package lme4 (Bates and Maechler 2009) with Poisson error-distribution with sex of the parent (2 levels; i.e. mother, father) and familiarity (2 levels, i.e. own and alien fledgling) as fixed effects. To account for effects of the time parents had to learn the call of their fledgling or changes in call characteristics between recording and playback, we included the interaction between familiarity and both number of days between fledging and playback (continuous, range: 4-15 days) and number of days between recording and playback (continuous, range: 0-8 days)as fixed factors. As random factors we included parent identity, own fledgling identity and stimulus bird identity. Given that latency cannot be scored when there was no parental response, we only used number of calls as a response measure for glms. The standard model diagnostics of non-normal errors, non-constant error variance and the presence of outliers were performed on each of the final models according to Fox (2002).

RESULT

Individual variation in fledgling distance calls

The cross-validated DFA on 493 calls from 84 fledglings revealed that 70.6% of all calls (348 calls) were assigned to the correct individual. The correct assignment rate was significantly higher than the 1.2% likelihood to be assigned to the correct individual by chance (binomial test: $p<0.001$). The DFA proves that calls provided sufficient individual identity information to be statistically distinguishable (for example calls see figure 4.2). In a next step, we investigated which parameters contribute to individual variation and provide potential for individuality coding. PIC, i.e. the potential for individual identity coding, showed values well above one for all acoustic parameters. Three out of 10 parameters show values above two (Table 4.1). High repeatabilities and high values for PIC demonstrate that distance calls of fledglings provide a high degree of individuality in all measured call parameters (Table 4.1). PIC values and repeatabilities were highly correlated ($R^2=0.79$, $F_{1,8}=34.56$, $p=0.0004$).

Table 4.1

Potential for individual identity coding and repeatabilities for ten acoustic parameters from 493 calls of 84 individuals.

		Potential for individual identity coding				Repeatabilities	
Acoustic parameters		mean±SD (untransformed)	meanCVi	CVb	PIC	R	Confidence interval
Duration (ms)		210.24 ± 62.66	17.56	35.53	2.02	0.83	0.81-0.86
Amplitude	mean	32.47 ± 4.83	21.60	40.70	1.88	0.85	0.83-0.89
Amplitude modulation (1/ms)	variance	$(5.06 \pm 2.56)\mathrm{x}10^{-3}$	24.90	45.86	1.84	0.82	0.80-0.85
Frequency (Hz)	mean	3933.65 ± 411.20	22.34	32.79	1.47	0.73	0.68-0.77
Frequency modulation	mean	11.78 ± 6.39	15.89	30.50	1.92	0.81	0.78-0.82
Frequency modulation	variance	286.62 ± 121.03	24.13	35.82	1.48	0.75	0.68-0.81
Entropy	mean	-2.51 ± 0.44	14.53	28.05	1.93	0.80	0.77-0.82
Entropy	variance	0.29 ± 0.15	28.75	35.76	1.24	0.66	0.63-0.71
Pitch (Hz)	mean	619.68 ± 66.53	13.06	35.37	2.71	0.88	0.85-0.90
Pitch goodness	mean	829.94 ± 314.53	18.66	40.62	2.18	0.86	0.82-0.86

The DFA on differences between broods of 43 genetic siblings from 14 broods showed a weak effect, 18.6% of fledglings were assigned to the correct brood, which is significantly different from a 7.1% chance of being assigned to the correct brood randomly (binomial test: p=0.01). The DFA on sex differences of fledglings showed that 53.6% of 84 individuals were assigned to the correct sex, which is equal to a by chance correct assignment rate of 50.0% (binomial test: p=0.59).

Vocal recognition of nestlings – a playback experiment

To test the ability of parents to recognize their own offspring acoustically, we tested 42 parents (21 breeding pairs) with calls of 64 fledglings (1-7 per brood). Three adults did not respond to any stimulus. Most responses to playbacks to either own or alien fledglings were single calls (Table 4.2). The response pattern for responses with only one call showed that calls were emitted specifically in response to the stimulus (median (Q125% / Q375%): own: 900ms (330ms / 2671ms); alien: 719ms (366ms / 2664ms); figure 4.3) and not in a random pattern, where average latency would be around 2500ms. The latency of an adult to respond to a stimulus was related to the number of response calls (Spearman rank correlation, r_s=-0.26, p=<0.0001, N=1205 responses). The number of response calls in response to the stimuli calls revealed that parents did not respond differently to own or alien fledgling (glm: b±SE=-0.03±0.06, t=-0.60, p=0.55, N=42, figure 4.4) nor did the sex of the parents affect the overall responsiveness (glm: b±SE=0.56±0.42, t=1.34, p=0.18, N=42, figure 4.4). The response pattern of adults was not related to the number of days the fledgling had left the nest (interaction familiarity x number of days fledged: glm: b±SE=0.02±0.03, t=0.53, p=0.59, N=42) nor was it related to the number of days between the recording and the playback (interaction familiarity x number of days between rec and playback: glm: b±SE=-0.03±0.04, t=-0.78, p=0.43, N=42).

Table 4.2

Counts of how often adults responded with a certain number of calls to stimuli from own or alien fledglings and the according percentage of the overall response. Responses for alien are three times higher than own, due to a 1 : 3 ratio of own versus alien stimuli per adult.

	Number of response calls per stimulus							
	0	1	2	3	4	5	6	7
own	456	224	63	19	5	1	0	0
	59.4%	29.2%	8.2%	2.5%	0.7%	0.1%	0.0%	0.0%
alien	1411	588	212	60	21	7	4	1
	61.2%	25.5%	9.2%	2.6%	0.9%	0.3%	0.2%	0.0%

DISCUSSION

The results of this study show that fledgling distance calls are highly individually distinct and can be discriminated statistically, based on their acoustic properties. Further, we demonstrate that adult zebra finches do call in response to playback of fledgling distance calls, however, they do not respond differently to the calls of their young compared to alien young.

DFA failed to discriminate the sex of fledglings using acoustic parameters from distance calls. This suggests that distance calls presumably contain very little to no information about the sex of a fledgling, at least when considering the acoustic parameters measured in the present study. Acoustic differences between broods were also not found in this study; the DFA on brood differences did not assign nestlings to broods correctly. This suggests that parents would most likely be unable to distinguish between their own and alien fledglings based on a common signature that all fledglings from one brood share. Consequently, parents would need to recognize their fledglings individually to discriminate them from foreign offspring, instead of using a brood signature for all their offspring.

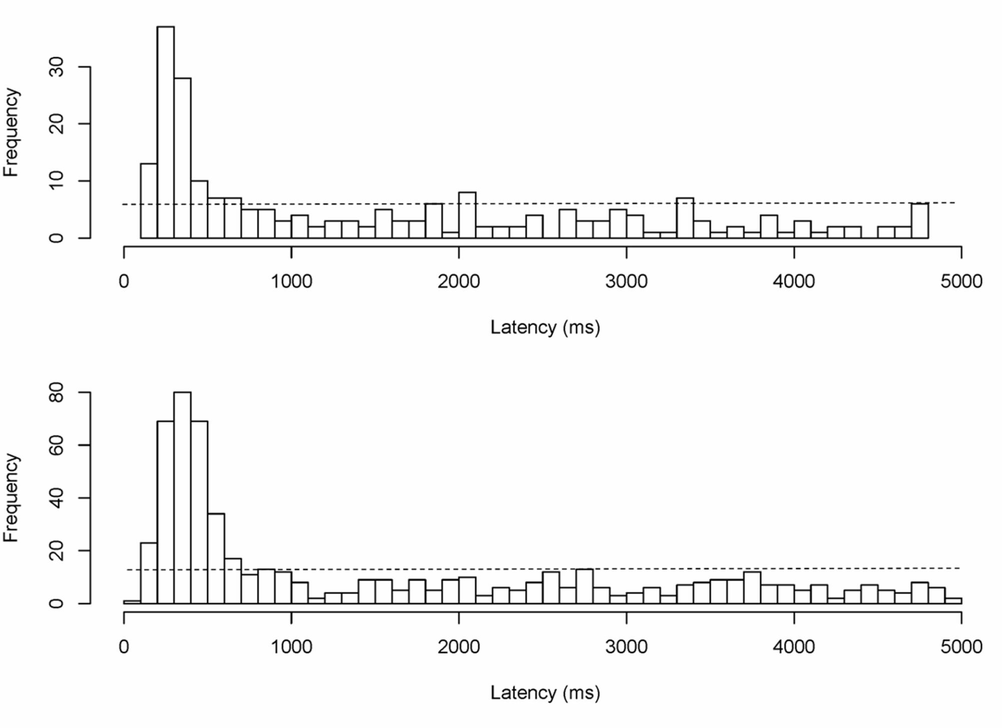

Figure 4.3
Histogram plots of single response calls in relation to latency of response to (A) own and (B) alien fledglings. Most responses are given shortly after the stimulus, which shows that calls are emitted in response to the stimulus. The dashed line indicates average response frequency of random response latency. Response frequencies for alien fledglings are three times higher, caused by a 1:3 ratio of own versus alien stimuli per adult.

Levréro et al. (2009) have shown that begging calls of young zebra finches contain individualized information one day before the nestlings leave their nest. In contrast to begging calls used in the study by Levréro et al. (2009), we have used distance calls of offspring shortly after fledgling. Those calls are more similar to the adult distance calls and consist of a single call compared to the begging call, which contains a train of calls and is only used in begging situations (Zann 1996; Levréro et al. 2009). The DFA on distance calls of young fledglings demonstrates that calls provide individual information sufficient to discriminate statistically between fledglings. The individuality in fledgling calls, as measured by the DFA, is more similar to distance calls of adult zebra finch females (i.e. 72%, N=94 females, using 10 acoustic parameters), but less than in distance calls of adult males (i.e. 95%, N=100 males, using 10 acoustic parameters) (Jacot et al. 2010). PIC values show that every measured parameter shows very high levels of individuality.

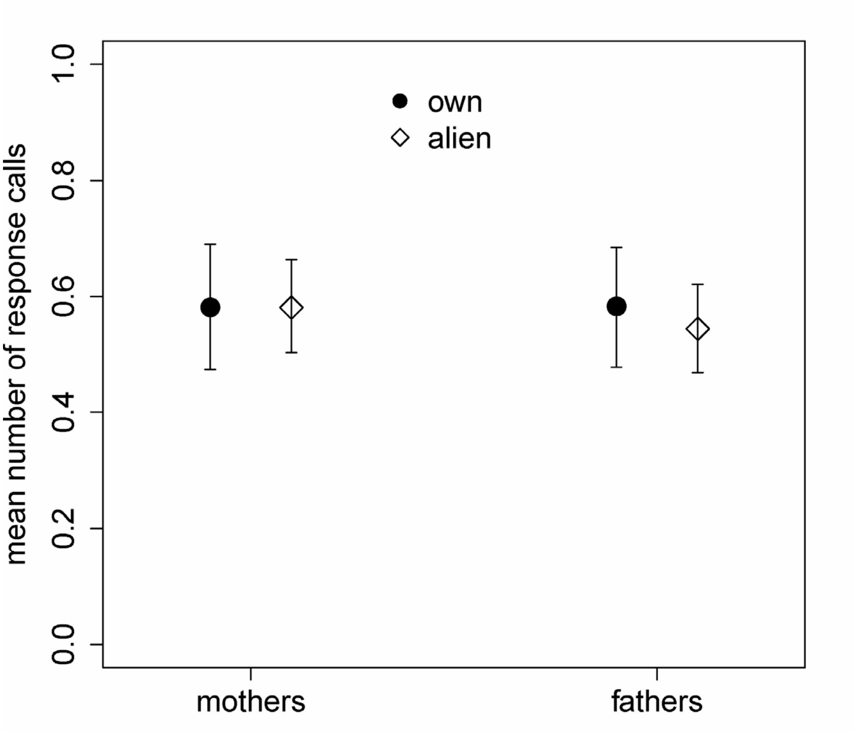

Figure 4.4
Average number of response calls (± standard errors) for mothers and fathers to own and alien fledgling calls.

In the playback experiment, parents responded specifically to stimulus calls and not in a random manner (Figure 4.3). However, despite the high degree of acoustic individuality in distance calls of fledglings, parents did not respond specifically to their own offspring. Do parents lack the perceptual abilities to distinguish between their own and foreign fledglings based on distance calls? Although some studies do not find parental recognition of chick vocalizations (e.g. Beecher et al. 1986; Benedict 2007; Bonal and Aparicio 2009), this seems unlikely for the zebra finch. Previous studies have shown that adult zebra finches are able to recognize mates using distance calls (Vignal et al. 2004; Vignal et al.

2008). Zebra finches even showed the ability to discriminate between individual humans based on the speakers voices (Ohms et al. 2010). Fledgling distance calls provide similar acoustic individuality to adult distance calls (see above), parents should therefore be able to perceive acoustic differences between fledglings.

It is possible that the lack of specificity in parental response is due to a lack of motivation in relation to the experimental set-up. Parents may not respond stronger in order to minimize the risk of the chicks getting into a dangerous situation. However, previous studies on parent-offspring communication and on the genetic basis of zebra finch vocalizations using the identical experimental setup have shown that zebra finches reacted in a more or less natural way (Forstmeier et al. 2009; Jacot et al. 2010). It remains possible that chick recognition shortly after fledging is primarily based on variation in begging. After fledging, recognition of distance calls in zebra finches may not be mutual but one-sided, and parent-offspring recognition may follow a two-step process. In a first step, fledglings recognize distance calls of their parents, respond with their own distance call (Jacot et al. 2010) and move in the direction of their calling parents. Once the fledgling is reunited with its parents, parents may use the fledglings obligatory begging display, emitted to solicit food, to acoustically recognize their fledgling and avoid costly false feeding (Levréro et al. 2009). This scenario conflicts with the high rate of false feedings reported by Jacot et al. (Jacot et al. 2010), which, however, might be a consequence of the close proximity of breeding pairs within the aviary situation. In the wild, Zann (Zann 1996) described that parents and fledglings use distance calls to reunite, but did not give details about the exact sequence of calling and approach behaviour. Studies in the wild or in larger aviaries allowing spatial segregation might be able to clarify the importance of begging and distance calls in parent-offspring recognition and parental feeding patterns.

Although we were able to demonstrate that fledgling distance calls show potential for individual recognition, we cannot conclude that distance calls are actually used by parents to recognize their offspring. This highlights the importance of adopting a signaller’s and a receiver’s perspective in a signalling system. Just demonstrating individuality in a signal does not imply that this information is used by the receiver for individual recognition. This study also points out, that complex social interaction might be altered by laboratory conditions and therefore might only be fully understandable in a more natural context. Further field and laboratory studies are clearly needed to understand the complexity of vocalizations and their functions in different social contexts.

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Chapter 5

Sticking out of the crowd: The effect of acoustic similarity on recognition

Hendrik Reers, Wolfgang Forstmeier, Alain Jacot

Abstract

Individual recognition has been shown in many taxa and social context. However, it is not yet well understood how distinctiveness of individuals is related to success or failure of recognition processes. Playback experiments have shown that individuals respond to 'false stimuli', suggesting that they mistake one individual for another. Distinctiveness might therefore greatly influence the precision of recognition processes. Here we used acoustic parent-offspring recognition in zebra finches to test whether acoustic similarity between individuals can influence recognition. In a playback experiment we presented parental calls and non-parental calls of known similarity to fledglings. Our results demonstrate that acoustic similarity influences the likelihood of recognition errors; with decreasing similarity, the likelihood of recognition errors decreases as well. We also show that recognition of parents by fledglings is very precise and that fledglings show baseline responsiveness to conspecific calls. In combination with the nearest-neighbour similarity distribution across the population of adults, this study suggests that distinctiveness from other individuals is likely to reduce recognition errors and therewith might provide a selective advantage for distinct phenotypes.

It is well established that individual recognition (e.g. through visual, olfactoral or acoustic signals) commonly occurs. In birds, the most common modality to convey information about the identity of an individual is via individual signatures in acoustic signals. An extensive body of literature demonstrates that acoustic signals in birds, and in other taxa, are individually distinctive and facilitate individual recognition (e.g. Jouventin et al. 1999; Charrier et al. 2001; Jacot et al. 2010). Individual recognition, and therewith individual distinctness, is necessary in a variety of repeated social interactions to ensure that investments reach the correct recipient. Selection for individual recognition is therefore expected to act on both the ability of the receiver to perceive differences between individuals and on the distinctiveness of individuals (Tibbetts and Dale 2007).

Distinctiveness is thought to be maintained through negative frequency-dependent selection, which promotes distinctive or rare signals/phenotypes (Dale et al. 2001; Sheehan and Tibbetts 2009; Sheehan and Tibbetts 2010). As soon as a 'rare' phenotype evolves, this might give the bearer a selective advantage through being more easily recognizable (Tibbetts and Dale 2007). This trait is then expected to spread, until the trait becomes common and a new rare phenotype evolves, resulting in big intra-specific variation in identity signalling traits (Tibbetts and Dale 2007). While negative frequency-dependent selection is expected to increase variation in the selected trait among the population, selection for distinctiveness is likely to be counterbalanced by the need for species recognition.

Concluding, one expects that acoustically very different individuals are easier to discriminate than very similar individuals (Dale et al. 2001). Surprisingly however, studies testing the effect of similarity on the precision of recognition processes, and thus the selective advantage of distinctiveness, are rare. A few studies on visual recognition signals indicate that recognition errors can be the result of differences in similarity. Guillemots (*Uria aalge*) recognise their own eggs when those are switched with neighbouring eggs, but tend to make more mistakes when the switched eggs are similar in appearance (Tschanz 1959). A similar link between similarity and recognition mistakes can be found in Northern masked weavers (*Ploceus taeniopterus*), in which the female is less likely to reject an added egg when it is similar in colour to her own egg (Jackson 1992).

To our knowledge there are no studies specifically testing whether similarity in acoustic signals predicts the likelihood of recognition errors.

In this study on captive zebra finches, we aimed to answer the question on how fledglings respond to playback in relation to acoustic similarity in adult calls. Zebra finches are opportunistic breeders with biparental care, nest in loose colonies of up to a hundred pairs and offspring are fed during an extended post-fledging phase by their parents (Zann 1996). Adult zebra finches of both sexes have been shown to recognize individuals of the other sex in a mate recognition context (Vignal et al. 2004; Vignal et al. 2008). It has also been shown that young zebra finches preferentially respond to parental calls, suggesting that young are able to recognize their parents acoustically (Jacot et al. 2010; Mulard et al. 2010). However, Jacot et al. (2010) have also shown that fledglings respond to non-parental calls and proposed that such responses to non-parents could be recognition errors, related to acoustic similarity between parents and non-parents.

We first measured all acoustic similarities between all individuals of the study population to design playbacks with known similarity between individuals. This also allowed us insight into the probability of being acoustically similar depending on where an individual is compared to the population mean. In a next step, we specifically tested if the response to non-parents depends on acoustic similarity to the parents' distance calls. We predicted that fledglings will respond mostly to parental calls, but that the response to non-parental calls will decrease with decreasing similarity to parental calls.

METHODS

Subjects and housing

Fledgling zebra finches used in the present study originated from a captive population held at the Max Planck Institute for Ornithology in Seewiesen, Germany. All fledglings tested in this study are descendants from birds breeding in aviaries that held six breeding pairs. The sex of the offspring was determined using molecular methods (Griffiths et al. 1998). Temperature in the rooms was maintained at 24±1°C and relative humidity ranging from 40 to 60%. Rooms were illuminated by full-spectrum fluorescent light (Osram Lumilux T5 FH 28W/860 Daylight) and the light:dark period was 14:10 h. All birds received a millet seed mixture, cuttlefish, grit, water *ad libitum* on a daily basis and a multivitamin supplement once per week. All recognition trials were conducted between May and August 2009. Aviaries were checked twice a day for newly fledged birds. Nestlings were individually marked by numbered alloy bands.

Acoustic distances between calls

We used calls of 100 male and 94 female zebra finches (number of calls/individual, mean±sd, males: 15.76±6.29, females: 10.53±1.71) to get a measure of population variability in call features. The calls were analysed with Sound Analysis Pro software 2.065 (Tchernichovski and Mitra 2004) using standard settings. We extracted the following acoustic features to characterize the acoustic variability of the population: 1) call duration (ms), 2) variance in amplitude modulation (1/s), 3) mean frequency (Hz), 4) mean frequency modulation, 5) variance in frequency modulation, 6) mean entropy, 7) variance in entropy, 8) mean pitch, 9) mean pitch goodness and 10) mean principal contour (for details see Tchernichovski and Mitra 2004). To investigate the effect of acoustic similarity on probability to respond we used stimulus calls with known similarity to parental calls. The acoustic similarity (i.e. acoustic distance) between calls were calculated for both sexes from separate linear discriminant function analyses (N males=100, N females=94) (package: MASS, Venables and Ripley 2002). Using the discriminant scores for each call, the centre for an individual (i.e. the centroid) was calculated as the mean of each discriminant scores for all calls from one individual. The 'representative call' for each individual was then defined as the call with the smallest Mahalanobis distance to its centroid (distances to centre of group; mean±sd: males: 0.86±0.44; females: 1.43±1.08). As a measure of acoustic similarities between individuals, we calculated the Mahalanobis distance between the representative calls of all individual, separately for each sex. For each individual we picked the five most similar calls (similarity category 1, rank 1-4 in distance, mean±sd male: 4.21±3.50, female: 4.65±2.77), five calls with intermediate similarity (similarity category 2, rank 40-44 in distance, mean±sd male: 11.72±5.73, female: 11.83±4.09) and five calls that were dissimilar (similarity category 3, rank 80-84 in distance, mean±sd, male: 19.35±6.73, female: 19.14±4.85) as stimulus calls for the playback experiment. We choose to limit the distance rank range to 84 out of 100, or 94 respectively, to avoid extreme individuals and outliers.

To investigate if acoustic similarity between parents is correlated to how similar nestlings of different broods are, we compared the distance of representative calls for fathers and mothers of 13 broods and the centroids of their respective broods with each other. Distances were calculated following the above methods of extracting Mahalanobis distances and centroids from discriminant function analysis on males, females and broods.

Playback protocol

We simulated a situation in which a fledgling lost visual and acoustic contact to its parents and will respond to distance calls of parents (Zann 1996; Jacot et al. 2010). We used 120 recently fledged chicks from 48 broods from 28 families ($N_{females}$=61, N_{males}=59; age: 24.7±1.1days; days fledged 6.7±2.0 days). Fledglings were tested singly in a sound-attenuated recording box (70cm x 50cm and 50cm high) which was equipped with a small metal wire cage containing a single perch, a microphone (C2, Behringer GmbH, Willich, Germany) approximately 20cm from the perch and a small loudspeaker (V20, Logitech, Morges, Switzerland) next to the microphone. The microphone was connected to a pre amplifier (SM Pro Audio, Melbourne, Australia) from which we recorded directly through a M-Audio Delta 44 (AVID Technology GmbH, Hallbergmoos, Germany) sound card onto the hard drive of a computer at a sampling rate of 44 kHz and 16 bit amplitude resolution using Audacity 1.3.7 (D Mazzoni, Canada, http://audacity.sourceforge.net/). Audacity was used to play back distance calls and to record the fledgling's reaction simultaneously.

The playback experiment started with 2.5 minutes of silence to allow for acclimation of the fledgling to the recording box (Jacot et al. 2010). For each individual stimulus bird we build a 30-second 'individual-unit' that consisted of the same call starting at 0, 5 and 10 seconds, followed by 20 seconds silence (see figure 5.1). Four of these stimuli, each from a different similarity category, were combined for each sex, resulting in a 2-minute 'sex-unit' for each sex. The order of individuals within a sex-unit was randomized. Two 'sex-units' per sex were alternately combined, resulting in an 8 minute total playback stimulus (i.e. male, female, male, female or female, male, female, male). The order of the sex was alternated between each recorded fledgling. Using a single call per individual will lead to inflated variance components of the random effects but will lead to correct test statistics of the fixed effects, hence our analyses do not face the problem of pseudoreplication.

The fledgling's acoustic response was measured as the number of calls within 5 seconds from the start of each stimulus call. In addition, we measured the latency to respond as the time from the start of the stimulus call to the fledgling's first response call. In cases where fledglings did not respond to a stimulus, the count of response call was 0 and latency could not be scored.

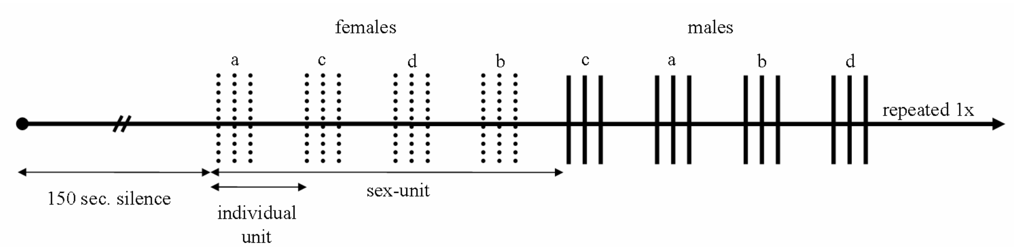

Figure 5.1
Playback design: 3 identical calls for 4 different adults per sex were used. The sex unit was altered and each sex unit was played twice with the individuals being in the same order.

Statistical analysis

All statistical analyses were performed with R 2.8.0 – R 2.10.0 (R Development Core Team 2008; R Development Core Team 2010).

To describe the distribution of acoustic similarity in relation to the position of an individual within the population, we calculated the linear regression of distance of an individual's representative call to the center of the population and the distance to the nearest neighbor for each individual. Both variables were log-transformed prior to analysis to approach normality.

To analyze the effects of stimulus similarity on the number of response calls and latency to respond we used generalized linear mixed-effects models (GLMM) (package *nlme*, Pinheiro et al. 2006). We performed two GLMMs, one on the differences between parents and the most similar individuals (i.e. category 1), the second one on differences between similarities of non-parents. As fixed effects, we used sex of both the fledgling and the stimulus birds as factors, and the similarity category as a continuous variable. As a random effect, we added fledgling identity to the model. The standard model diagnostics of non-normal errors, non-constant error variance and the presence of outliers were performed on each of the final models according to Fox (2002).

RESULTS

The acoustic distance to the nearest neighbour and the average distance to other individuals within the population depend on the position of an individual within the ‘acoustic space’ of the population. For individuals of both sexes the acoustic distance to the nearest neighbour (*linear regression*; females: 0.51±0.08, $F_{1,92}=45.0$, $p<0.0001$, N=94 (Fig. 2a) males: b±SE=0.60±0.07, $F_{1,98}=74.8$, $p<0.0001$, N=100 (Fig. 2b)) and mean distance to all other individuals (*linear regression*; females: 0.43±0.02, $F_{1,92}=355.7$, $p<0.0001$, N=94 (Fig. 2c) males: b±SE=0.46±0.02, $F_{1,98}=616.7$, $p<0.0001$, N=100 (Fig. 2d))increases the further the individual is away from the population mean

Effect of call similarity on response pattern

In the playback experiment 100 out of 120 individual fledglings ($N_{females}=51$, $N_{males}=49$) responded to stimuli at least once. Those 20 ($N_{females}=10$, $N_{males}=10$) trials in which fledglings did not respond were discarded from further analysis. Of the 100 fledglings that did respond, nine responded to males only, nine to females only. Two fledglings out of 100 responded to parental calls only, while six fledglings responded to unrelated adults only. From the 94 fledglings that did respond to parental stimulus calls, 16 fledglings responded to their fathers only, 12 to their mothers only.

Fledglings reacted more (GLMM: b±SE=-0.22±0.02; z=11.03; $p<0.0001$; N=100; figure 5.3) and faster (GLMM: 414.7±63.1; z=6.56; $p<0.0001$; N=97; figure 5.3) to parental calls than to calls of the most similar non-parents (i.e. similarity category 1). In a second step we analysed a fledgling’s response towards non-parental calls in respect to call similarity. The similarity of non-parental calls affected the response pattern of fledglings. Fledglings responded less (GLMM: -0.057±0.009 calls/similarity step; z=6.47; p<0.0001; N=100; figure 5.3) and slower (GLMM: 176.2±39.5 ms/similarity step; z=4.46; p<0.0001; N=98; figure 5.3) to non-parental calls that are less similar to parental calls. For all models there was no effect of either sex of the fledgling on the response pattern (all p>0.22) nor did the sex of the stimulus birds affect the reaction of the fledglings (all p>0.64).

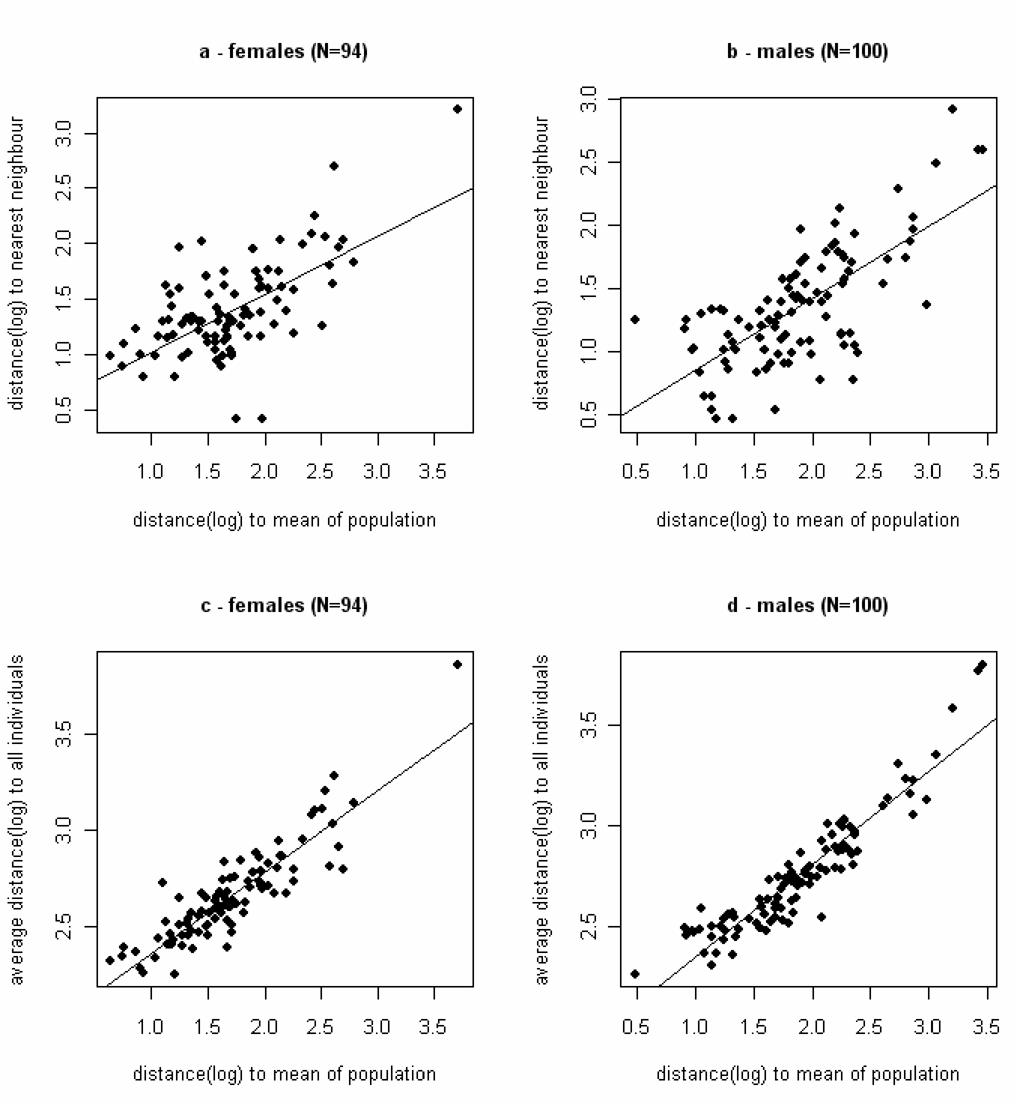

Figure 5.2

Relationship between the acoustic distance of an individual to the mean of the population and both its distance to its nearest neighbour (a and b) and the average distance to all other individuals within the population (c and d) for both sexes. All distance measures are log-transformed.

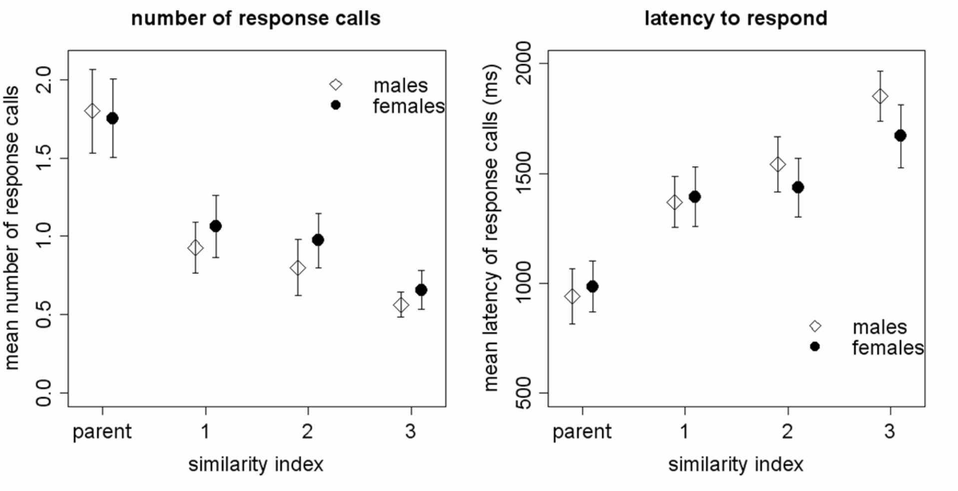

Figure 5.3

Mean number of response calls for fledglings of both sexes in response to parental calls and increasingly dissimilar non-parent calls (1-3).

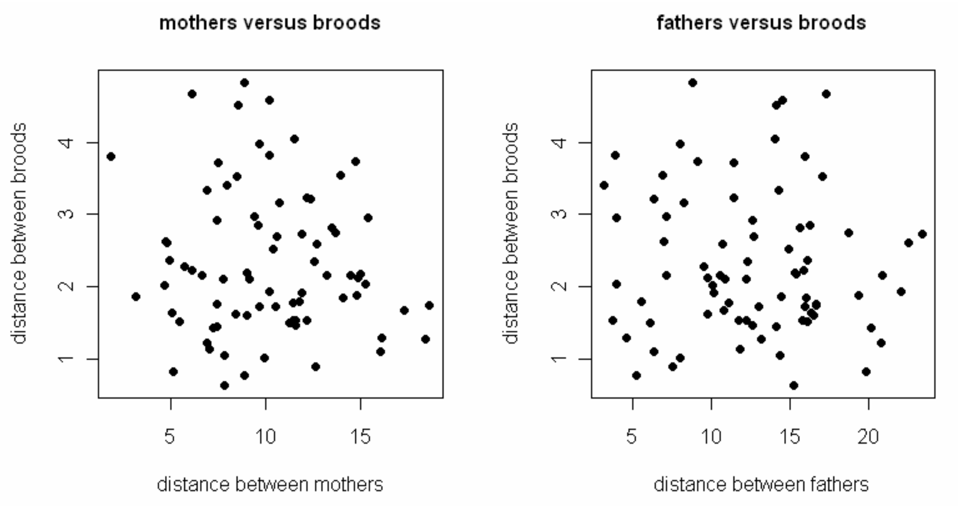

Figure 5.4

Distance between all mothers and father from 13 broods and their respective distances between the centroids of their broods. Centroids of broods are based on a DFA on broods using representative calls of 40 chicks. No relationship between similarity of adults and broods.

Call similarity between parents and among their offspring

Acoustic similarity between fathers or mothers did not predict the similarity between respective offspring (*linear regression*; fathers: b±SE=-0.01±0.02, $F_{1,76}$=0.31, p=0.56, N=78; mothers: -0.03±0.03, $F_{1,92}$=0.69, p=0.41, N comparisons=78, see figure 5.4).

DISCUSSION

Our study demonstrates that young zebra finches recognize their parents and delivers an explanation why fledglings respond to unrelated adults. Previous studies have already indicated that fledglings often respond to contact calls of unrelated conspecifics (e.g. Sealy and Lorenzana 1997; Jacot et al. 2010). However, it remained largely elusive why and to whom offspring respond. Here we provide first experimental evidence that similarity in an acoustic signal affects the response pattern in the receiver. Our study demonstrates that the propensity of a fledgling to respond to non-parental calls is positively linked to the similarity to its parents' calls.

When presenting calls of varying similarity, fledglings reacted stronger to calls that were more similar to their parents' calls. This effect was independent of the sex of the offspring and the effect of similarity on response behaviour was similar towards fathers and mothers. A stronger response towards adults that sound similar to their own parents could be due to a fledgling's strategy trying to solicit food from unrelated adults or fledglings committing recognition errors. Under the strategy-scenario, a fledgling would try to 'cheat' on other adults, who mistake the responding fledglings for their own. This scenario assumes a relationship between adult call similarity and call similarity among their offspring. There was no indication for such a relationship in our large dataset (see figure 5.4).

The response pattern to non-parental calls was consistent with the predictions from the recognition error hypothesis. This hypothesis states that the likelihood of committing a recognition error depends on the similarity to the individual to be recognized (in our case the parents). A fledgling's response decreased with decreasing acoustic similarity to its parents' calls (see figure 5.3). These results indicate that fledglings do not follow a strategy in order to obtain food but that recognition errors can largely explain the occurrence of 'false responses' towards non-parents.

Although the responses decreased with reduced similarity of non-parents to parents, fledglings still responded to the least similar calls. The responses to those very dissimilar non-parental adults may still partly represent recognition errors but may additionally reflect baseline responsiveness to conspecifics. Baseline responsiveness may be beneficial in a social context, for example to maintain contact within a flock (Zann 1996). In conclusion, the response pattern towards unrelated adults may herewith incorporate components related to recognition errors and some baseline responsiveness.

Fledglings' responding towards unrelated adults has been reported in several species and our study is now delivering an explanation about the mechanism why these false responses happen. Under the assumption that false responses are potentially costly in terms of attracting predators (e.g. Briskie et al. 1999; Lima 2009) and given that adults are known to attack unrelated offspring that solicit extensively for food (Proffitt and McLean 1990; Hauber 2002) we predict that variation in call signatures will spread whenever there is strong selection for individual recognition. High variation in call signatures will facilitate recognition processes since average call similarity to conspecifics is reduced. In a comparative approach, Loesche et al (1991) have found that species breeding in large colonies and where the risk of confusion is high show larger variation in call signatures than species breeding in small colonies or solitarily, a result in line with the predictions from our experimental results.

Adopting an individual's perspective, birds at the acoustic outer boundaries of a population are less likely to have similar neighbours and on average show a bigger dissimilarity to other individuals within the populations (see figure 5.2). Again, whenever misidentifying is related to evolutionary costs, one expects a selective advantage of individuals that are very different to the mean of their population (Tibbetts and Dale 2007). Under these conditions, negative frequency-dependent evolution can shape the signal properties and lead to an increase in signal variability within a population or species (Sheehan and Tibbetts 2009). Our study shows an underlying mechanism of this evolutionary process that might be widespread in many taxa and may partly explain the observed variation in call signatures of our zebra finch population.

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General discussion

In this dissertation, I set out to investigate the evolution of identity signalling systems focusing on parent-offspring recognition in birds. The combination of studies of my thesis provide novel insights for several aspects of parent-offspring recognition specifically and identity signalling in general. First, I adopted a signaller's perspective and investigated proximate mechanism promoting variation in the signal itself. I was able to provide first experimental evidence on how identity signals and condition signals (i.e. hunger) are incorporated into one signal (Chapter 1) and how acoustic parameters are influenced by a chick's genetic background and the growing environment (Chapter 2). These results demonstrate that certain call parameters are shaped by short-term changes in hunger and the growing environment of a chick, while others are unaffected by hunger level and are largely determined by the chick's genetic background.

In the second part of my thesis I adopted a receiver's perspective, and tested whether parent-offspring recognition is mutual (Chapter 3 and 4) and investigated proximate causes for 'false responses', i.e. responses to unrelated adults (Chapter 3 and 5). Our results indicate that only fledglings readily recognize their parents. The results further demonstrate that fledglings sometimes respond to unrelated adults and that false responses reflect recognition errors, which are most common in relation to female contact calls and are related to the acoustic similarity between call features. Below I will review our results, point out unanswered question and suggest new directions for future research on identity signalling.

Signallers and their signal

Influences on the signal

Parents are thought to learn the calls of their offspring while the chicks are still in their nests to ensure reliable recognition once they are fledged. However, due to changes in hunger, begging calls are constantly varying. These short-term changes in begging calls are expected to complicate the accurate learning of a chick's individual signature in the begging or contact call. In the first chapter we investigate the question how begging calls can incorporate information about a chick's hunger while still preserving an individual signature necessary for accurate recognition by their parents.

Whereas behavioural measures of need have been studied extensively (e.g. Redondo and Castro 1992; Kilner 1995; Kilner et al. 1999), only few studies have focused on changes in acoustic parameters (e.g. Marques et al. 2008; Marques et al. 2009). Our study was the first to investigate the effect of hunger on acoustic parameters and the resulting consequences for a chick's individual signature. On the level of acoustic parameters we were able to show that only a few parameters are largely unaffected by hunger while most parameters changed with hunger (Reers and Jacot 2011). However, hunger-related within-individual changes in call parameters were relatively low compared to between-individual differences (Reers and Jacot 2011). The results of this study indicate that acoustic individuality is maintained even over changes in hunger and therefore allows parents to learn their offspring's acoustic signatures (Reers and Jacot 2011).

Potential consequences of higher between- compared to within-individual differences in call characteristics are discussed in the section “Is there more to the signaller?” below.

The first chapter has demonstrated high individuality in call characteristics. However the underlying sources creating these differences among individuals remained unclear. In the second chapter, I investigated potential sources of variation due to a chick’s genetic background and growing environment. Here, we investigated the influences of genetic and environmental effects on specific acoustic parameters in the contact calls of tree swallows. Since avian vocalizations can contain both learned (e.g. Sharp et al. 2005) and heritable (e.g. Medvin et al. 1992) components, a cross-fostering experiment was the technique of choice to separate effects due to a common origin (i.e. genes and maternal effects) and common rearing environment (i.e. nest conditions and learning). While we found a clear brood signature in untreated nests, cross-fostered nestlings did not show a common brood signature, neither for their nest of origin nor for their rearing nest. On the level of acoustic parameters, some parameters were strongly determined by the nest of rearing, others by the nest of origin. Duration and entropy are the main parameters affected by the nest of rearing, suggesting that nestlings adjust these call parameters to each other. This finding supports previous findings in tree swallows that show that calls of nestlings become more similar when they call together instead of solitarily (Leonard et al. 2009). The only parameter that was largely determined by the origin of a chick was frequency modulation.

What is special about frequency modulation?

Frequency modulation (FM) seems to play a special role for acoustic individual recognition. Playback experiments have demonstrated that FM is an important call parameter for individual recognition in zebra finches(e.g. Lengagne et al. 2001; Jacot et al. 2010), penguins (Lengagne et al. 2000) and in seals (Charrier et al. 2001). We can support the importance of FM for recognition in zebra finches. In chapter 3, 4 and 5 we found that in both adult and fledgling zebra finches FM contributes to differences between individuals (Jacot et al. 2010).

In chapter 1, we were able to show that FM is largely unaffected by changes in hunger (Reers and Jacot 2011), making it an acoustic trait that can be used for recognition despite changes in hunger state. Furthermore, the results of chapter 2 show that FM is the only acoustic trait that is largely determined by the origin of a nestling. Effects of nest of origin most likely reflect genetic effects and to a lesser extent maternal effects. These finding gives indirect support for the importance of frequency modulation for individual recognition processes and indicate a heritable component of FM, suggesting that differences in FM could be the result of negative-frequency dependent selection.

Why is FM important for individual recognition? This may be explained by the virtually endless complexity that can be created in the pattern of a sound by modulating frequency over time (Figure D.1). The more complex the general structure of the time-frequency pattern of a call is, the more possibilities there are for a unique signature (Figure D.1). The examples calls in figure D.1 illustrate that calls of weaver birds (first row) and tree swallows (second row) show a more complex time-frequency pattern with much more changes in frequency than calls of zebra finches (third row). One can imagine that the highly complex structure of the former two species allows more unique FM patterns than zebra finch calls.

Is there more to the signaller?

In chapter 1 we investigated how two different types of signals are incorporated into one call. Contradictory to our initial predictions, call parameters were not categorized into static identity signals (see A in Figure D.2) and dynamic hunger signals (see B in Figure D.2). Instead we find that all acoustic parameters that changed with hunger also showed a high degree of individuality. Consequentially, the absolute signal value is an unreliable signal of need due to large between individual differences (see C in Figure D.2). If the receiver estimates hunger on an absolute scale, parental care is not distributed equally to all chicks, but mostly to the one with the highest signal value; a problem that is especially eminent in species with asynchronous hatching, as found in our weaver bird study system. A solution to this problem and a way to precisely estimate an individual's state of need or hunger is to perceive signals on a relative scale. Parents would first need to know the identity of a chick and in a next step estimate the hunger from the relative signal value within the chick's acoustic range - the signal value is referenced to the acoustic range of the signaller.

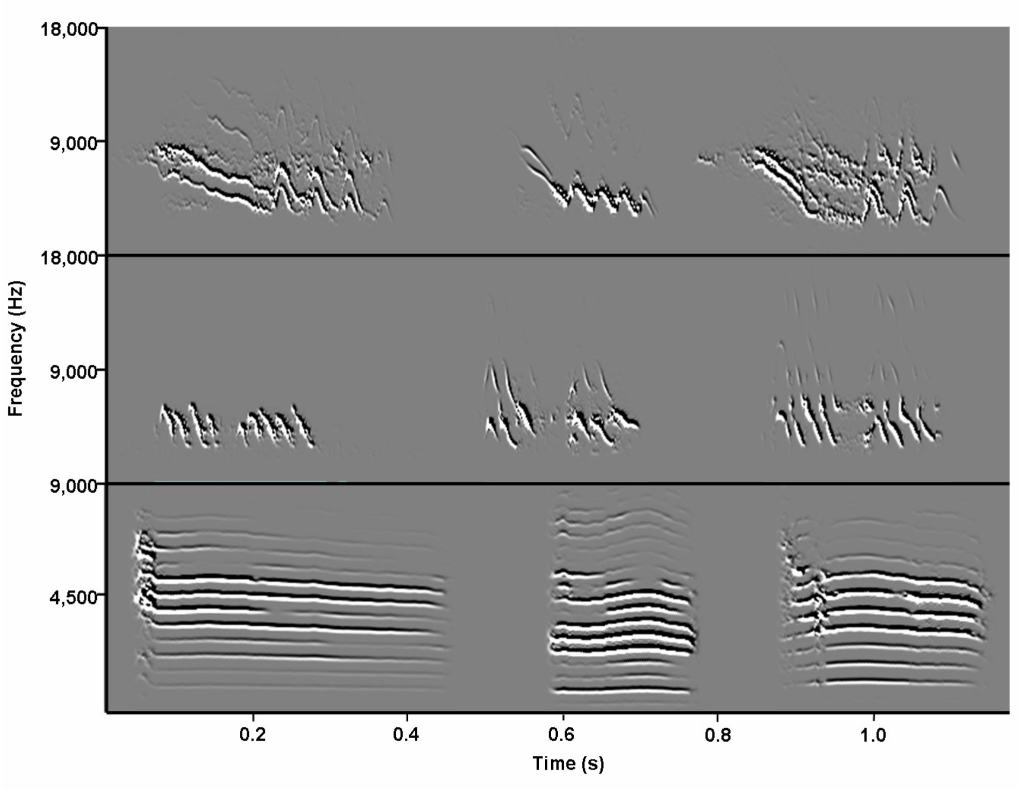

Figure D.1

Representative calls of young of the three studies species of this dissertation illustrate the intra- (across) and interspecific (down) differences in call structure. (Top) The first row show calls of three nestlings of Jackson's golden-backed weaver at the age of 13 days. (Middle) The middle row shows calls of three tree swallows nestlings close to fledging at the age of 20 days. (Bottom) The last row shows calls of three zebra finch fledglings of 25 days of age. In comparative approaches, Medvin et al. (1993) and Loesche et al (1991) have found that species breeding in large colonies and where the risk of confusion is high show more complex calls than species breeding in small colonies or solitarily. Of my study species, the weaver is the most colonial species. Tree swallows and zebra finches are both semi-colonial, but tree swallows show the tendency to gather in large roost shortly after fledging.

This is the first study to suggest such a mechanism for the perception of condition dependent signals. Traditionally, condition-dependent signals were thought to be estimated by the receiver on an absolute scale (e.g. Iacovides and Evans 1998; Candolin 2000). This is likely to be the case whenever individuals only meet once and are not able to know an individual's acoustic range. However, in repeated social interactions (e.g. in group living species or parental care) there is potential that familiarity between individuals can largely increase efficient communication.

The only studies that I am aware of and that suggest the importance of acoustic familiarity with an individual for the estimate of identity unrelated information are on distance estimates of songs in great tits (*Parus major*) (McGregor and Krebs 1984) and eastern meadowlarks (*Sturnella neglecta*) (McGregor and Falls 1984). Males in these species can only estimate the songs of neighbours when they are familiar with their neighbour's songs. From this finding the authors suggested that males have an acoustic template of their neighbours' songs and use this to compare a just heard song with this template to obtain an estimate of sound degradation, which is used as a proxy for distance.

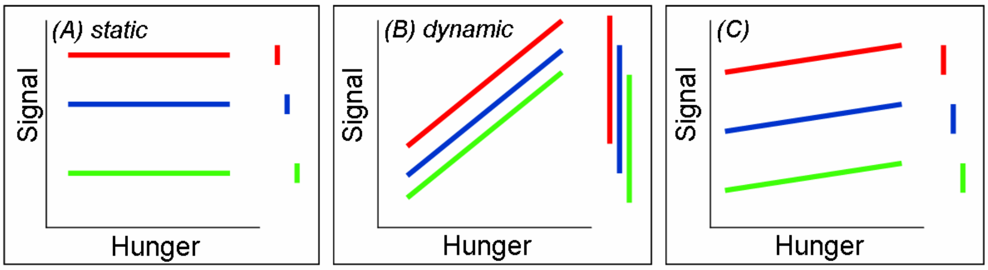

Figure D.2
Schematic explanation of different signal properties over changing condition (e.g. hunger) for three individuals (red, blue and green lines). A) To ensure consistent individual identity information one would expect that signals do not change with hunger and therefore do not overlap in trait properties. B) Hunger signals are expected to change with hunger and therewith reliable signal the sender's condition. Additionally one expects that competition between individuals will lead to directional selection an absolute hunger signal and result in overlapping signal ranges between individuals. C) A combination of a dynamic signal of hunger with reliable individual identity information of the sender. As a consequence the absolute hunger value is not a reliable indicator of hunger due to huge between individual variations. Instead, to precisely estimate the condition of the sender, the receiver can reference the signal to the within-individual signal range of the sender.

How familiarity with an individual's signal range can affect the perception of information about a sender is a process that every person experiences when they compare communication with familiar and unfamiliar persons. With experience of the signalling behaviour of a person, one becomes able to read subtle emotional changes that are unreadable for inexperienced persons but might be of importance for social interactions.

While the effect of familiarity in human communication is rather intuitive, this ability is underappreciated and research largely absent in other species and communication systems. Individual-referenced signalling could be a widely used communication component in social communication systems, which allows learning of acoustic signal templates through repeated interactions. Future research on this aspect of identity signalling might discover exciting cognitive abilities in animals, with implications on our understanding of complex communication in social groups.

The receiver's perspective

One of the questions of my PhD was whether selection for distinctiveness in colonial species can increase variation between individuals. For distinctiveness to provide a selective advantage, recognition needs to be beneficial and recognition errors are expected to imply costs. In the case of parent-offspring communication false responses increase calling and therewith increase the risk of attracting predators (e.g. Briskie et al. 1999; Lima 2009). Also, false investments by parents that provide parental care to unrelated young are expected to be costly, not only for the parent as a receiver, but ultimately also for the young as a signaller (e.g. Mulard and Danchin 2008). Under the assumption that recognition errors are costly, we can use recognition errors as a proxy for selective benefits of rare signatures. However, the biological relevance of this benefit is still unclear and it remains untested whether this benefit could translate into a real fitness advantage that could promote the spread of rare individual signatures.

In chapter 3 we found that fledglings not only responded to their parents, but also to unrelated adults. We argued that these responses might either be explained by recognition errors, caused by similarities between individuals, or represent a strategy to cheat on unrelated adults. To test if fledglings are able to cheat on unrelated adults, we tested the ability of parents to recognize their fledglings by contact calls (Chapter 4). An earlier study on parent-offspring recognition in zebra finches had already shown that parents are able to recognize their young acoustically; however, this study used begging calls of nestlings close to fledging (Levréro et al. 2009). Interestingly, in our study parents did not respond preferentially to their own fledglings. This finding is surprising since adults are able to recognize their mates by contact calls and should therefore have the perceptual means for recognition (Vignal et al. 2004; Vignal et al. 2008). It remains possible that parents did not respond to calls of their fledglings to avoid attracting them into a potentially dangerous situation created by our playback setup. The results of chapter 4 were not conclusive about whether responses to unrelated adult are a cheating strategy to take advantage of the inability of adults to recognize their young or whether responses of fledglings to unrelated adults represent recognition errors.

The 'false responses' of chicks towards unrelated adults in chapter 3, stimulated me to design an experiment trying to disentangle whether 'false responses' reflect a chick's strategy or indeed recognition errors. In chapter 5, we used playback experiments with adult contact calls to measure if the outcome of communication is related to the acoustic similarity between individuals. We predicted that recognition errors occur more often when the calls of two individuals are very similar compared to individuals that are acoustically distinct. Our results confirmed this prediction, ruled out the strategy hypothesis, and provide first experimental evidence that the likelihood for recognition errors is related to similarities in acoustic identity signals.

Within the same study we also examined how acoustic similarity is distributed throughout a population. We found that the further an individual is placed from the mean of the signal space (i.e. the multi-dimensional space over all acoustic parameters), the bigger the average acoustic similarity to its nearest acoustic neighbours.

Under these conditions, negative frequency-dependent selection can shape the signal properties and lead to an increase in signal variability within a population or species (Sheehan and Tibbetts 2009). This selective mechanism will likely drive the evolution for distinctiveness not only in acoustic signals, as in our studies, but also in signals of other sensory modalities such as visual or olfactoral cues.

Unanswered questions and future directions

Understanding variation in identity signals

The experimental studies of my thesis have contributed to the understanding of proximate causes for variation in acoustic individual signatures. While there is ample evidence that signals used in parent-offspring communication and other social interactions are highly individualized (e.g. Peake et al. 1998; Reby et al. 1998; Mathevon et al. 2003), experimental evidence for the proximate causes of phenotypic variation used as identity signals is still scarce. We need approaches using quantitative genetics and field experiment (e.g. cross-fostering experiments) investigating the effects of additive genetic variation or maternal effects on single call characteristics within identity calls. Additionally, an important source for acoustic identity traits on the level of group or kin recognition is learning (e.g. Sharp et al. 2005). However, the ability to use learned vocalizations for recognition is hardly studied (Sharp et al. 2005).

We have to be aware that demonstrating high individuality and potential for individual recognition does not imply that this information is used in recognition processes. Neutral selection, i.e. whenever there is no selection against a newly occurring trait, will also promote variability in this trait and it is essential to adopt a signaller's and a receiver's perspective to understand the adaptive significance of variation in signal value.

In addition, future research should focus on where the limitations for the evolution of signals of identity are? So far, I argued that an increase in phenotypic variation can increase recognition and therefore be beneficial. However, there are possible limitations to the evolution of identity signals. Most signals are used in a multitude of contexts, ranging from species recognition, male-male competition and female mate choice to predator avoidance. One can imagine that extreme phenotypic variation can lead to failure of species recognition or be detrimental in terms of sexual or natural selection.

How does recognition work in detail?

Actual empirical evidence of recognition is rare and often does not go beyond a yes/no answer for its occurrence (e.g. Lovell and Lein 2005; Levréro et al. 2009).

This is unfortunate, since our results demonstrate that recognition errors, i.e. responses to signals from 'wrong' senders, provide an elegant way to get insight into an individual's ability to recognize signals. This ability may depend on multitude of factors (e.g. personality, condition or social context) and future research could use these recognition errors for understanding fine-scaled variation in recognition. In addition, using recognition errors is an effective way for testing hypothesis about the importance of single signal characteristics for individual recognition. By experimentally changing single signal parameters (e.g. acoustic parameters, visual traits or chemical components of odour signals) one can use the occurrence of recognition errors to investigate the importance of single signal parameters.

It remains also largely unknown how between-individual differences are processed on a cognitive level. Template matching is likely to be the most important step in any recognition process (McGregor and Krebs 1984). When perceiving a signal, the receiver can match this signal with an acquired template for the according information (e.g. species, sex or individuals). However, unknown is how and when templates are acquired and whether such a process is permanent or flexible. Research in this field is likely to not only reveal novel insights on adaptations for individual recognition but more generally also on cognitive abilities of animals to make associations between perceived signals and previously acquired information.

Conclusion

With this dissertation I was able to provide new insights into the functions and evolution of identity signals. Individual recognition is a prerequisite for repeated interactions and as such a highly important skill for communication in any social species, including our own. The findings of my PhD contribute to our understanding of the evolution of identity signals and hopefully raise further questions in this fascinating field of research.

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Author contributions

Chapter 1

H.R. contributed with concept, study design, practical work, data analysis and writing; A.J. with study design, practical work and writing.

Chapter 2

H.R. contributed with concept, study design, practical work, data analysis and writing; A.J. with discussion and writing; M.L.L. with concept, study design and study population; A.G.H. with study design, practical work and discussion.

Chapter 3

H.R. contributed with concept, study design, practical work, data analysis and writing; A.J. with concept, study design, practical work, data analysis and writing; W.F. with concept, study population and discussion.

Chapter 4

H.R. contributed with concept, study design, practical work, data analysis and writing; A.J. with concept, study design and writing; W.F. with concept, study population and discussion.

Chapter 5

H.R. contributed with concept, study design, practical work, data analysis and writing; A.J. with study design and writing; W.F. with concept, study population and study design.

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