Species recognition in zebra finches: testing the effects of sex, sensory modalities, and social ontogeny

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Abstract

Species recognition is an integral component of mate selection and must occur in all sexually reproducing organisms to avoid costly hybridisation. Species recognition abilities may be comprised of both innate components and experience during ontogeny through the learning of visual, acoustic, and other sensory species-specific cues. But how greatly is the ability to recognise one's own species (conspecifics) over others (heterospecifics) dependent on the phylogeographic relationship of the array of potential species as social partners and to what extent is the discriminatory behaviour modulated by subject ontogeny versus species identity? Using a model system, which is widely studied in all disciplines of avian research, the zebra finch (*Taeniopygia guttata castanotis*), I aimed to investigate the visual and acoustic cues involved in conspecific recognition by both female and male individuals of this species. I used an array of previously untested phylogeographically relevant estrildid heterospecifics as my stimuli and tested subjects of diverse experimental ontogenetic treatments.

By scoring a wide-selection of measured behavioural responses my research indicates that female and male zebra finches prefer live conspecifics over live phylogeographically relevant heterospecific stimuli and this preference is more consistent by females than males. Female zebra finches rely on both visual and acoustic features of potential social partners for accurate species discrimination; in this regard video playbacks or the diverse colour morphs of domesticated zebra finches may be useful tools for further experimentation. Additionally, females display significant individuality in their behavioural responses which may be relevant for pair bonding decisions made by both sexes. I further documented that normally-reared zebra finches will prefer song playbacks of their own species but that both rearing in an indoor restricted acoustic environment of conspecifics or cross-fostering to another species will reduce discrimination preferences, although the results may depend on the behavioural metrics analysed.

This dissertation is presented as a general overview with details of my specific contributions towards the work included in this thesis, followed by discrete review and data chapters, and a final general discussion.

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General overview

1.1 Researching species recognition in zebra finches in the laboratory

Behavioural ecology is the study of the ecological and evolutionary bases for variation in animal behaviour, answering proximate and ultimate questions of why animals behave the way they do. The laboratory setting enables the isolation and control of specific variables, the removal or randomisation of confounding factors and simplifies the tracking of an individual's behaviour. Laboratory experiments, in parallel and in comparison to field studies, are valuable for answering specific questions and certainly most ecological investigations can benefit from a combined experimental approach.

My thesis focuses on a prolific area of behavioural ecological research, namely species recognition in the model species, the zebra finch *Taeniopygia guttata*. Using laboratory experiments and the unique information that these can provide, I provide an overview and novel insights into three aspects of zebra finch species recognition systems: sex differences, sensory modalities and ontogenetic effects. I then make general conclusions based on my data and review and suggest future avenues for research.

The analytical portion of my thesis begins with a focus on conspecific recognition, which is essential for sexually reproducing species. Captive zebra finches are a model system in which the behavioural, ontogenetic, and neurobiological bases of own-species (conspecific) recognition have been studied in detail. To assess the potential role of phylogeographic effects on species recognition, I examined the spatial preferences of unmated captive-bred female zebra finches between unfamiliar captive males of conspecific and estrildid heterospecific male stimuli. In accordance with prior studies using domesticated Bengalese finches (*Lonchura striata* vars. *domestica*), I found significant spatial and behavioural preference for conspecific males by female zebra finches, irrespective of heterospecific male phylogeographic origin, mating status or individual behaviour. This result has ramifications for the interpretation of social and mate preferences in this model species as it implies a consistency of species discrimination by captive female zebra finches.

Although captive zebra finches are a model system for studying the factors involved in species recognition and mate choice, to date male zebra finches' behavioural responses in a spatial preference paradigm to a range of estrildid finch species, other than domesticated Bengalese finches, remain unknown. I investigated spatial and display responses of male

zebra finch subjects to stimulus females between conspecific and four phylogeographically relevant finch species, in addition to female Bengalese finches. Surprisingly, male subjects did not show consistent spatial association with conspecific over heterospecific females. Overall, as predicted by sexual selection theory, the spatial proximity responses of males were less discriminatory compared to female zebra finches' responses tested previously using the same paradigm. However, male subjects showed consistently more behavioural displays towards female conspecifics than heterospecifics which were positively related to the behavioural display rates of the respective female stimuli. Some male behavioural responses, other than song, also showed significant differences between the different stimulus species and consistently differed across individual test subjects, with the most individual subject variation seen in choice trials between female conspecific and Bengalese finch stimuli. The results are again important for the design and interpretation of future behavioural and neurobiological experiments on species recognition systems using the zebra finch as a model species.

Based on these initial experiments, I set out to study in detail the multimodal species recognition cues of zebra finches, using playbacks of visual and/or acoustic traits that can be valuable tools for investigating their relative importance of various sensory modalities in the recognition process. Specifically, I investigated captive-bred females' behavioural responses to video playbacks of male conspecific or phylogeographically relevant heterospecific cues. Females showed consistent spatial discrimination between sympatric estrildid finch taxa when acoustic and visual cues were presented together or acoustic only cues were played back. In contrast, females showed no discrimination when visual cues only were available and when the acoustic cues of stimulus species were experimentally swapped or mismatched. These results are consistent with acoustic cues being more salient than visual cues, available from video-playbacks, for zebra finches. Subjects showed significant individual behavioural variation in their 'attentiveness' to playback stimuli. The consistency of this behavioural individuality was confirmed by a positive correlation between a female's attentiveness and whether it became pair bonded with an unfamiliar male in a free-flight aviary paradigm. These results also suggest that consistency in behavioural variation in female zebra finches may be relevant in both species recognition and pair bonding contexts and further contribute to the factors that lead to appropriate mate selection decisions in the face of ecologically relevant variation in con- and heterospecific stimuli and sensory cues.

Given my findings that female zebra finches use visual and acoustic traits for accurate recognition of male conspecifics, I set out to collect experimental evidence of the individual roles of these cue types affecting conspecific recognition. In a spatial paradigm to test discrimination, I used live male zebra finch stimuli of two colour morphs, wild-type (conspecific) and white with a painted black beak (foreign), producing one of two vocalisation types; songs and calls learned from zebra finch parents (conspecific) or cross-fostered songs and calls learned from Bengalese finch (*Lonchura striata* vars. *domestica*) foster parents (foreign). I found that female zebra finches consistently preferred males with conspecific visual and acoustic cues over males with foreign cues, but did not discriminate when the conspecific and foreign visual and acoustic cues were mismatched. These results confirm prior findings regarding the importance of both visual and acoustic features for female zebra finches when discriminating between live conspecific males.

In birds in general and in zebra finches in particular, it is clear that vocalisations are critical sensory cues for species recognition in most bird species. Conspecifics during development provide the most reliable of sensory cues for species recognition in parental bird species. The zebra finch (*Taeniopygia guttata*) is a sexually dimorphic model species used for investigations of the features of the behavioural cues and neurobiological substrates of species recognition. Regarding acoustic conspecific cues, theory predicts that exposure to both con- and heterospecific vocalisations and other environmental sounds results in more accurate auditory species discrimination because diverse vocal cues during development shift optimal conspecific acceptance thresholds to be more restrictive to yield fewer acceptance errors. I tested the behavioural preferences of female and male zebra finches raised in an outdoor environment (Control) and female and male zebra finches reared in an indoor colony with exposure to zebra finches only (Restricted), to playbacks of songs of zebra finches, zebra finches cross-fostered by Bengalese finches (*Lonchura striata* vars. *domestica*), and Bengalese finches. Several behavioural measures revealed minimal sexual dimorphism in discrimination but showed that Control subjects preferred conspecifics' songs over either the songs of cross-fostered zebra finches or Bengalese finches. Restricted zebra finches in contrast did not discriminate behaviourally between the three song types. These results support the concept of a shift in the species acceptance threshold in the Restricted treatment resulting in more acceptance errors. I discuss future work to test the role of exposure to diverse vocal cues of both con- and heterospecifics in the ontogeny of song perception in this important laboratory model species for social recognition research.

The final component of my thesis concerns song-production, -discrimination, and –preferences in oscine birds which are dually influenced by both species identity and the ontogenetic environment. Specifically, the cross-fostering of progeny of zebra finches into heterospecific nests of the Bengalese finch allows for a further exploration of the sensory limits of early development and the effects of species-specific acoustic cues upon song discrimination in adulthood. To quantify the song preferences of female and male normal-reared (control) and Bengalese finch fostered zebra finches, I recorded multiple behavioural measures, including spatial proximity, vocalisation rates and response latency, during sequential song-playback choice-trials using both tutor species' songs and the songs of two other ecologically relevant Australian species, the owl finch (*Taeniopygia bichenovii*) and the star finch (*Neochmia ruficauda*). Response strength was variable between the different measures, but no differences were detected within the specific behavioural responses towards the song playbacks of the two sexes. Control subjects strongly preferred their own species songs while Bengalese-fostered zebra finches exhibited reduced song discrimination between con-, tutor-, and heterospecific songs. Overall behavioural responsiveness was also modulated by social ontogeny.

Taken together, the results of my thesis indicate a difference in the strength of preference for conspecific stimuli that is dependent on the species identity, sex differences, and the complexity of the rearing environment in oscine birds and illustrate the role of multiple measures and the selection of ecologically relevant stimulus species in behavioural research using zebra finches.

1.2 Overview of thesis structure and contributions

Chapter 2 is based on material by Dana L.M. Campbell, Susan A. Weiner, Philip T. Starks & Mark E. Hauber in Annales Zoologici Fennici (in press). My contribution to this chapter was to write the first full draft of this work and to coordinate submissions from the other contributors. Specifically, the introduction, conclusion, literature review, and all sections on vertebrate examples of laboratory model systems for behavioural ecological research are my own original work, including customary minor comments on content and style by my thesis supervisor on the final draft.

Chapter 3 is based on material by Dana L.M. Campbell, Rachael C. Shaw & Mark E. Hauber in Ethology (2009). My contribution included the design of the study, the collection of nine-tenths of the data included, carrying out all statistical analyses, writing the first draft, incorporating comments from the additional contributors, and producing the final draft.

Chapters 4-8 are based on material by Dana L.M. Campbell & Mark E. Hauber in Behavioural Processes (2009), Animal Biology (submitted), Journal of Comparative Psychology (in press), Journal of Ornithology (resubmitted), and Animal Cognition (2009). My specific contributions included the design of the studies, the collection of all of the data included, carrying out all statistical analyses, and writing the first, subsequent and final drafts, incorporating customary minor comments on content and style by my thesis supervisor.

The General Conclusions are solely my work and outline considerations of the implications, short comings, and directions for future research that are based on my thesis. It incorporates customary minor comments on content and style by my thesis supervisor on the final draft.

2

Context and control: behavioural ecology experiments in the laboratory

2.1 Introduction

Behavioural ecology is a popular and active field of research that employs integrative approaches to study both the ecological and evolutionary bases for animal behaviour. Behavioural ecology endeavours to determine the role of behaviour in enabling individuals' adaptations and constraints to the surrounding environment (Krebs & Davies 1997). The initial research and observations of behaviourists (ethologists and comparative psychologists) demonstrated that though highly variable, certain behavioural patterns can also be exceptionally characteristic and descriptive at the level of each species (Lorenz 1937; Tinbergen 1963; von Frisch 1974). Together with the development of sophisticated theory and extensive experimentation this has led to the development of the thriving field of behavioural ecology, which connects individual and phylogenetic variation in behaviours with ecological variables and evolutionary history (Krebs & Davies 1997).

In behavioural ecology experimentation, researchers aim to answer four focal types of questions, as famously coined by Tinbergen (1963), and divide research areas into two major fields: proximate and ultimate causes of behaviour. Proximate questions look directly at the *causal* and *ontogenetic* factors that influence observed behaviours, whereas ultimate questions, in turn, are concerned with the *functional* adaptations and *evolutionary* processes that may have imparted a selective advantage to a certain behavioural trait (Tinbergen 1963; Krebs & Davies 1997; Drickamer et al. 2002). These questions are essential focal points for behavioural ecologists seeking a complete understanding of any and all behaviours observed in nature and examined in the laboratory.

Field research on animals in their natural environment can provide the most appropriate and richest source of information on both the relationships of organisms to individual environmental variables and the relationships between multiple organisms within specific environmental parameters (Simberloff 2004; McGill et al. 2006; Johnson & Stinchcomb 2007). However, it is clear that in the field setting there are also countless external variables beyond the realm of experimental control that may be influencing the particular target behaviours we wish to study. It is in this regard that we take organisms into a laboratory setting to facilitate a more extensive control over external environmental and, potentially, internal physiological and motivational variables, as well as limiting the number of interacting species or individuals that can all impact on behavioural outcomes. Of course a

captive approach does limit the interpretations and applicability of results when the target species is isolated from influencing factors that may be present in a natural field situation (Table 2.1). Therefore, the chosen research setting of field vs. laboratory must ultimately depend on the question to be answered, although undoubtedly many research topics in animal behaviour can benefit from a combination of both laboratory and field techniques. Accordingly, when we surveyed the literature of experimental techniques used in all published articles over 12 months of both '*Animal Behaviour*' and *Behavioral Ecology*' (July 2006 - July 2007) we found similar proportions of studies that occurred solely in the laboratory (including experiments on captive populations in reasonably natural enclosures) or solely in the field (research on wild animals which may or may not have been handled by investigators to individually mark/tag for identification) and very few studies that looked at the focal animals' behaviour in both settings (Fig. 2.1).

Here we will focus on discussing varying settings and methods used solely in the laboratory under the realm of several major types of behavioural ecological sub-disciplines that animal behaviourists are actively engaged in investigating. We present both vertebrate and invertebrate exemplars and discuss what these methods uncover and what possible limitations the laboratory environment places on interpretation. We also discuss three varying laboratory research contexts that may be employed, outlining their main advantages and disadvantages. The methods and topics presented here are not in any way exhaustive, rather they highlight the range of techniques currently available in a laboratory setting for application in behavioural ecological research.

Table 2.1: The costs and benefits of laboratory experiments in comparison to field studies with both vertebrate and invertebrate examples provided.

Laboratory Experiments vs. Field Studies	
*The **Benefits** of laboratory experiment in comparison to field studies*	*Examples*
Controlled settings allowing the manipulation of environmental variables.	Controlling water quality to modulate chemical cue concentrations in Tilapia (Gonçalves-de-Freitas et al. 2008).
Experimental control for testing the influence of single factors only.	Quality of artificial ant nests (Pratt 2005).
Controlled social development for captive-reared animals allowing assessment of innate responses and effects of social learning.	Tool-making in New Caledonian crows (Weir et al. 2002; Kenward et al. 2006).
Isolation and tracking of individual development and testing of reactions at an individual level.	Assessing effects of varying ontogeny on the quality of male zebra finches as perceived by female conspecifics (Spencer et al. 2003, 2005; Naguib & Nemitz 2007).
Ability to limit or exclude conspecific and/or heterospecific interactions.	Isolation of newly emerged *Polistes* females (Shellman & Gamboa 1982).
Ease of physiological manipulation for testing specific reactions.	Altering diet quality to assess effects on plumage characteristics and song development in zebra finches (Naguib & Nemitz 2007).
*The **Costs** of laboratory experiment in comparison to field studies*	*Examples*
Laboratory strains of animals are often domesticated and therefore may behave differently from their wild counterparts.	Physiological condition and mate preference behaviour in zebra finches (Ewenson et al. 2001; Rutstein et al. 2007).
Artificial food supply can alter foraging behaviour.	*Ad libitum* food sources lead animals to engage in more costly behaviours (Kotiaho 2001).
Captive environments may increase subject and stimuli stress levels modulating behavioural results.	Mate preferences in zebra finches (Roberts et al. 2007).
Artefacts of the experimental environment can influence behavioural choices.	Leg bands and lighting conditions in zebra finch mate preference trials (Cuthill et al. 1997; Hunt et al. 1997).
Specific experimental results can be context-dependent and the unnatural laboratory environment can potentially obscure their observation.	Studies of kin recognition in the paper wasp, *Polistes dominulu* (Starks et al. 1998).

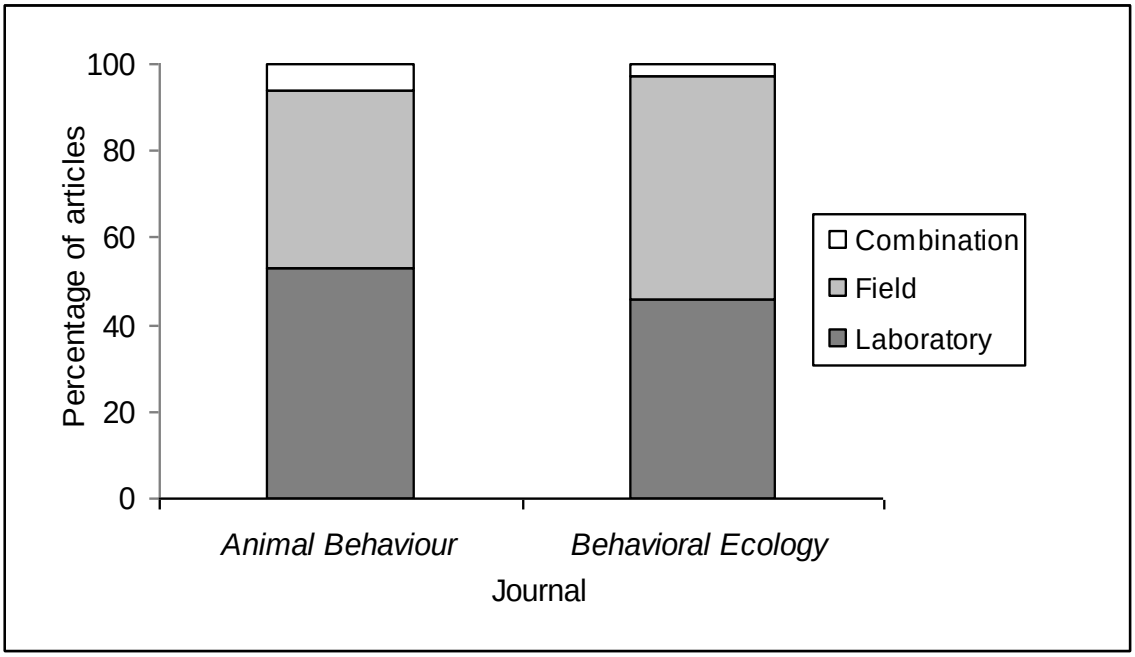


Figure 2.1: The percentage of surveyed experimental articles from *Animal Behaviour* (n = 246) and *Behavioral Ecology* (n = 208) that were carried out in the 'Laboratory', 'Field' or a 'Combination' of Laboratory and Field settings. Although similar in overall proportions, *Behavioral Ecology* published more field only and fewer lab and combination studies ($X^2 = 6.34$, $df = 2$, $p = 0.042$).

2.2 Four sample questions in behavioural ecology research

Within the field of behavioural ecology, researchers can set out to answer questions within several major areas of animal behaviour that can be explored in the laboratory setting. We selected studies that fall into the four categories of mate selection, nepotism, foraging and dominance. We will present representative examples that highlight the techniques that may be employed when researching these areas, as well as their limitations.

2.2.1 Mate selection

Mate selection in animals is a well-studied area of behavioural ecology research. This area is focused on understanding the factors or recognition facets, such as correct species, sex, genetic relatedness and genetic quality that can lead an animal to select one individual over another for mating and/or pair bonding (Sherman et al. 1997). Laboratory mate selection research endeavours to dissect the specific features of individuals that may make them attractive or unattractive as potential mates, and to identify what exact perceptual, physiological, and behavioural processes are involved in reaching the final mating decision. Through examples taken from a model system, the zebra finch (*Taeniopygia guttata*) (Zann 1996), we discuss here the use of live stimuli, highlighting findings and limitations of the laboratory setting, including impacts of experimental artefacts and the influence of proximal environmental factors on subject ontogeny.

The zebra finch is a small, sexually dimorphic, socially monogamous, Australian passerine. Mate preferences are frequently assessed via the presentation to a subject individual of a selection of live stimuli birds that vary to different degrees in phenotypic or behavioural attributes (Zann 1996), such as bill colour (e.g., Collins et al. 1994) or variations in song rate (Houtman 1992). These preferences for one individual over another (or several others) in a simultaneous choice scenario are frequently measured by the proportion of choice time spent with a certain stimulus individual (reviewed in Forstmeier & Birkhead 2004), a measurement of passive choice (cf. active operant choice: e.g., Riebel 2000). This passive spatial association method is widely used across different choice experiments (Hauber et al. 2000) but is not without interpretational constraints; the use of a sole preference metric and a lack of physical interactions between subject and stimuli can restrict determination of sexual or

social subject motivation (Rutstein et al. 2007). The passive choice paradigm does not require context-specific action by the individuals beyond spatial movement, which occurs throughout everyday activities (Hauber et al. 2001) and for highly social birds such as the zebra finch, choice could just be indicative of a wish to associate rather than indicating an actual sexual preference (Riebel 2000).

An alternative method to assess preferences is by measuring multiple behaviours displayed by the subjects that are naturally produced in specific contexts, such as female or male courtship displays (e.g., Burley et al. 1982; ten Cate 1985; Clayton 1990; Collins et al. 1994; Mansukhani et al. 1996) and may reveal social, sexual, or pair bonding preferences that spatial associations fail to show (Chapter 4). A further methodological option for confirming sexual motivation is to allow interactions between subject and stimuli in a free-flight aviary paradigm and use detailed behavioural observations to document sexual pair bonds formed or ultimately, breeding success (e.g., Burley 1981; ten Cate 1985; Mansukhani et al. 1996; Rutstein et al. 2007). Most studies though would benefit from a combination of assessment methodologies or a validation of the behavioural metric used in a series of studies to be able to comprehensively measure responses to specific traits in addition to confirming subject motivation and choice context (Rutstein et al. 2007).

In addition to mate selection based on varying phenotypic and behavioural traits, artefacts of the experimental environment may also impact on choice decisions. Examples include human alterations of the study species' appearance, such as the application of leg-bands that significantly alter zebra finch attractiveness (Cuthill et al. 1997), or factors such as the lighting environment where the absence of ultraviolet wavelengths important in avian vision may influence natural mating preferences (Hunt et al. 1997). The physiological stress hormone levels of both the subjects and the stimuli can also influence female choice behaviour, which is important to consider in the handling of subjects and giving time to acclimatise to novel testing environments (Roberts et al. 2007). However, physiological measures of stress responses, for example after the experimental separation of pair bonded mates, could in some cases provide critical insights into behaviourally cryptic discrimination abilities of individuals (Remage-Healey et al. 2003).

Furthermore, it is important to consider how proximate environmental factors may influence subject and stimulus ontogeny. For example the nutritional and stress-related hormonal

environment can decrease the quality of males by altering ornamental plumage and song complexity, making them unattractive to females (Spencer et al. 2003, 2005; Naguib & Nemitz 2007). A poor developmental history of stimuli, for instance, may influence zebra finches' selections, irrespective of the phenotypic trait being experimentally manipulated, and poor developmental history of subjects may bias the strength, consistency or direction of their preferences based on their self-perceived poorer quality (Burley & Foster 2006). These factors indicate critically important knowledge of developmental history when testing for mate preferences in a laboratory setting. Finally, it must be considered that although laboratory environments permit experiments not otherwise possible in the field, captive environments may affect the physiology and behaviour of research animals leading to different responses than what would be found in their wild counterparts (e.g., Ewenson et al. 2001; Rutstein et al. 2007).

Laboratory settings, with zebra finches and with many other species, are ideal for measuring individual subject response to specific varying traits that cannot otherwise be assessed in the field, but can present difficulties in determining subject motivation. Additionally, the artificial experimental environment may modulate behaviours that would be present in the study species' natural habitat, which needs to be considered for result interpretation.

2.2.2 Nepotism

Natural selection acts on all living organisms, selecting for alleles and causing them to increase in frequency between subsequent generations. While selection typically occurs on individual survival to reproduction and the success with which individuals pass their own genes on into the next generation, non-descendant kin also share genes with the focal individual in proportion to their relatedness. Therefore, natural selection can select for individually costly behaviours that are preferentially directed at and favour relatives, as long as the benefit to kin (adjusted for relatedness) outweighs the cost to the focal individual (Hamilton 1963, 1964). In order for kin selection to act, individuals must be able to direct their aid to kin over randomly related (i.e., non-kin) conspecifics in the population. Within the laboratory setting, experiments can be performed to determine whether individuals can and do distinguish kin from non-kin, how they do so, and under what circumstances they show behavioural discrimination (Mateo 2002).

To direct aid towards kin, an individual must first be capable of reliably recognising or associating with kin. Individuals can be given choices with options that allow them to aid or harm partners that are kin or non-kin, allowing the researcher to discern what features are used for kin recognition by removing or altering cues. In addition, the laboratory setting allows for detailed manipulation of the context of interactions. Frequently, animals exhibit different levels of kin-discrimination in different experimental contexts (reviewed in Liebert & Starks 2004) and these manipulations allow researchers to determine what external variables can affect kin-discrimination.

Neutral arena trials are frequently used in the laboratory to detect kin recognition. Two or more individuals are placed in an arena with minimal natural cues then either resources (e.g., rove beetle larvae *Aleochara bilineata*: Lize et al. 2006) or threats (e.g., salamander *Hemidactylium scutatum*: Harris et al. 2003) are added to observe competition or defence behaviour that favours related individuals.

Studies with contrived contexts can be used to test how nestmate-recognition (a correlate of kin recognition) ability is acquired, as well as the mechanisms and contexts of recognition. To test the context dependence of recognition, proximate environmental cues such as nest fragments can be introduced to arena trials to observe the interactions that result (e.g., paper wasp *Polistes dominulus*: Starks et al. 1998). The learning of kin-recognition can be tested by removing very young animals such as paper wasps (*Polistes fuscatus*), from their parental nests upon emergence then rearing them in controlled isolated environments (e.g., single boxes), and subsequently assessing their nestmate recognition abilities (Shellman & Gamboa 1982). These animals may also be reintroduced to unrelated conspecifics or nest material to determine whether they learn those cues from their environment and discriminate in favour of their "adoptive" kin (e.g., *Polistes carolina* and *P. fuscatus*: Pfennig et al. 1983).

In colony-living organisms, kin discrimination can be tested by introducing animals to laboratory colonies (e.g., ant *Pachycondyla luteipes*: Kikuchi et al. 2007). By varying the animals introduced (e.g., Kikuchi et al. 2007) or the circumstances of the colonies to which they are being introduced (e.g., ant *Linepithema humile*: Vásquez & Silverman 2008), the researcher can determine the contexts that lead to acceptance and rejection of nestmates and non-nestmates. These laboratory studies have provided insight into the adaptive value of kin-

recognition by increasing our understanding of when animals do and do not display kin-discrimination.

One major flaw with laboratory studies of nepotism is that kin-recognition and discrimination behaviours are frequently context dependent (e.g., Liebert & Starks 2004). In some species, kin-recognition can be as simple as treating all conspecifics in the colony or nest as kin (reviewed in Holmes & Sherman 1982). Additionally, recognition behaviours are often affected by the social environment (e.g., Fletcher & Blum 1983) or by situational cues (e.g., Starks et al. 1998). Therefore, given that context can be influential, the unnatural environment of the laboratory can potentially obscure important aspects of kin discrimination or even prevent it from being observed at all (e.g., Starks et al. 1998).

One method of partially addressing the lack of an appropriate fitness context in laboratory studies is to augment laboratory kin recognition studies with parallel experiments in the field (Kikuchi et al. 2007). Nestmate discrimination studies can be supplemented by studies of relatedness (e.g., ant *Proformica longiseta*: Seppä et al. 2008) and aggression (e.g., *Polistes fuscatus*: Gamboa et al. 1991) in the field to determine whether animals are discriminating in the contexts and ways that are predicted. In addition, context can be varied within the laboratory setting (e.g., Starks et al. 1998) to test for potential effects of a more natural context. Especially when combined with field studies and genetics, laboratory studies can provide valuable insight into the adaptive value and ontogeny of kin discrimination.

2.2.3 Foraging

All living organisms require food for energy to survive, and evolutionary optimal foraging theories predict decisions to be made during foraging that maximise energy intake (Drickamer et al. 2002). There is an extensive variety of foraging tests that can be carried out in a laboratory setting to investigate food preferences, foraging strategies, and the development of foraging proficiency. We present here select examples from research on New Caledonian crows (*Corvus moneduloides*) of how behavioural ecology laboratory research can be used to enhance field findings and provide greater insight into the development and learning of foraging techniques.

New Caledonian crows are omnivorous, forest-dwelling corvids endemic to New Caledonia where field observations have shown these birds to be highly specialised in their manufacture of stick and hooked or barbed tools (exclusively from *Pandanus* spp. plants) that are used to pry out prey from holes (Hunt 1996; Hunt & Gray 2002). To expand on field findings, researchers in a controlled laboratory setting have specifically explored the development of tool use, the influences of social context, and the limitations of the tool use behaviour. Behavioural observations, through the tracking of a particular individual over time, have documented rapid *de novo* shaping and use of novel material (e.g., a piece of straight wire) to obtain food (Weir et al. 2002) in the absence of extensive prior experience in tool shaping, thus demonstrating spontaneous tool-making behaviour (Weir et al. 2002). Further developmental research revealed that laboratory-reared juveniles with controlled ontogeny and no prior experience, observation, or other means of social transmission of information, spontaneously used tools to obtain food (Kenward et al. 2005), which provided evidence of an innate predisposition for the tool-use behaviour.

The captive environment did, however, severely restrict sample sizes (e.g., Weir et al. 2002, one naïve individual of the two captive subjects) which limited the conclusions able to be drawn. In addition, the effects of the surrounding environmental conditions that would be present in the crows' natural habitat but missing in a laboratory setting must be taken into consideration. Field observations revealed that preferential stick or *Pandanus* tool use are highly specialised at the level of the individual (Hunt & Gray 2007) and between different locations (Hunt & Gray 2003). These levels of variability are consistent with a degree of niche partitioning and social transmission important for the cumulative evolution of the tool-use behaviour that is not accounted for in a laboratory setting, which may modulate behavioural patterns (Hunt et al. 2007).

Furthermore, while there is evidence for a predisposition for stick tool manufacture, there is little complimentary evidence for innate recognition of the *Pandanus* plant (Kenward et al. 2005) over other potential tool materials, which has implications for substrate use when investigating tool manufacture in the laboratory and differing strengths in the role of social learning.

To investigate in detail the possibility of social transmission of information and effects of learning, the full ontogeny of the tool-use behaviour was documented (Kenward et al. 2006).

Specifically, juveniles trained with human demonstrators later showed greater tool-use proficiency than the naïve individuals and preferred to handle objects they had previously seen being handled by their human trainers (Kenward et al. 2006). These and other findings suggest the specialised tool-using behaviour presents a partially inherited basis followed by individual learning and social influences to perfect tool formation and use (Kenward et al. 2005, 2006; Hunt et al. 2007) of which the full extent of social behavioural modulation is likely impossible to document in a captive environment.

Such laboratory experiments demonstrate the type of ontogenetic knowledge that can be gained about complex foraging strategies from socially isolated individuals and controlled rearing environments. Nonetheless, the natural ecological context has to be considered and how the artificial environment may modulate results and limit interpretations. Foraging studies such as these presented can benefit from a combination of both laboratory and field studies to have a greater contextual understanding of the observed behaviours.

2.2.4 Dominance

Many social animals exhibit dominance hierarchies that can be mutually beneficial through reducing conflicts among members of social groups involving the distribution of food (e.g., Appleby 1980), opportunities for reproduction (e.g., Blatrix & Herbers 2004), or the delegation of communal tasks (e.g., Tentschert et al. 2001). Since dominance hierarchies mediate factors that are necessary for survival and reproduction (e.g., Pagel & Dawkins 1997), the methods by which dominance hierarchies are established and maintained are important for understanding social behaviour in many animals. Within the laboratory setting, both the initial establishment of dominance hierarchies and the interactions within established dominance hierarchies can be observed. The value of dominance to an individual in a hierarchy can also be determined by manipulating environmental or group factors to see whether individuals choose to accept subordinate status, challenge for dominance, or leave the group.

Experimental trials with two or more individuals grouped in a neutral arena, and allowed to contest for dominance (e.g., paper wasp *Polistes dominulus*: Tibbetts & Dale 2004, Tilapia *Oreochromis niloticus*: Gonçalves-de-Freitas et al. 2008) allow researchers to isolate environmental predictors of the hierarchy structure such as chemical cues (e.g., Gonçalves-

de-Freitas et al. 2008) or predictors of dominance in individuals such as female mated status (e.g., *Polistes fuscatus*: Downing 2004).

The establishment and maintenance of dominance hierarchies can be investigated by creating contrived situations within the laboratory and observing the response of the animals. Animals with different traits, such as varying genetic strains, can be grouped in artificial colonies (e.g., rats *Rattus norvegicus*: Ely et al. 1997) or given the opportunity to found their own colonies (e.g., *Polistes fuscatus*: Downing 2004). These studies can allow researchers to determine the effects of specific changes in context on the foundation of dominance hierarchies (e.g., rats *Rattus norvegicus*: Duncan et al. 2006).

The fitness component of dominance can also be studied in the laboratory by modifying the social environment within a group and observing the changes in the dominance hierarchy. This can involve removing the dominant individual(s) and observing the re-establishment of the dominance hierarchy after their removal (e.g., ant *Dinoponera quadriceps*: Monnin & Peeters 1999, *Polistes dominulus*: Strassman et al. 2004). The dominant individual(s) may then be replaced to observe how they fare in the newly established dominance hierarchy (e.g., ant *Harpagoxenus sublaevis*: Bourke 1988). In addition to behavioural metrics, physiological measures such as sympathetic nervous system activity can be taken from individuals of differing dominance statuses to assess specific physiological correlates of being dominant or subordinate (Ely et al. 1997). Additionally, other modifications can be made to the nest or the environment such as removing reproductive-destined eggs in eusocial paper wasps to observe the effects on aggression in the dominance hierarchy (Reeve & Nonacs 1992).

Laboratory experiments allow for detailed manipulations and observations of social structures that are rarely possible in the field. However, laboratory experiments can exclude contextual cues that are important for the establishment and maintenance of a dominance hierarchy. Partners chosen by researchers are unlikely to be the same partners that would have been paired in the field, and may have different interactions. Laboratory studies that may have lacked important cues can be supplemented by replicating the observations in field studies of early dominance contests (e.g., *Polistes dominulus*: Dapporto et al. 2006) and by genetic studies of dominance in established colonies (e.g., paper wasp *Polistes annularis*: Peters et al. 1995). When these considerations are taken into account, however, laboratory

studies and the creation of unnatural experimental situations can allow observations on and provide information about the establishment and maintenance of dominance hierarchies that cannot be gathered in field studies.

2.3 Laboratory contexts

Within a laboratory setting, behavioural experiments can be executed in any of three contexts: neutral, natural or contrived. While no laboratory experiment can perfectly replicate field conditions, the different contexts vary in the amount of control they offer and in how well they relate to field conditions.

A neutral context is an experiment in which the animal is removed from natural cues and placed in a simple neutral arena. While in the arena, the animal has very few cues outside of those specifically provided for the experiment. These experiments are artificial and far removed from natural conditions, but permit close observation, fine control of variables and response to selected stimuli that are expected to provoke a reaction including, for example, a conspecific, a food item, or a predator cue.

Neutral arena trials with conspecifics can be used to study a variety of behavioural systems such as mate choice (e.g., cockroach *Blattella germanica*: Lihoreau et al. 2007; parasitic wasp *Aphidius ervi*: Villagra et al. 2007), kin selection (e.g., halticine bee *Lasioglossum erythurum*: Kukuk & Crozier 1990), dominance hierarchies (e.g., *Polistes dominulus*: Tibbetts & Dale 2004; *Oreochromis niloticus*: Gonçalves-de-Freitas et al. 2008) or food preferences (e.g., crayfish *Cherax destructor*: Meakin et al. 2008). They can also be used to study the mechanisms (e.g., Gravel et al. 2004), ontogeny or evolutionary history (e.g., Kukuk & Crozier 1990) of behavioural traits by presenting carefully controlled stimuli. But with the arenas being artificial environments they lack the variety of cues that animals may normally use, and may themselves provide cues that modulate the animal's behaviour. In addition, they remove context that may be important for making decisions, such as the distance to home or other resources, or the presence of predators. As such, they may not detect behaviour that would naturally be present (e.g., Starks et al. 1998) or may cause behaviour that would not normally occur (Weir et al. 2002). In order to compensate for the artificial nature of the environment, neutral arena trials should be followed with trials in more

natural environments to confirm their findings. Despite these concerns, neutral arenas allow for very tight control over many variables, and can tease apart effects of interwoven factors.

A natural context experiment, in comparison, is an attempt made within the laboratory to replicate some aspect of a natural environment as much as possible. While a laboratory environment can never truly replicate field conditions, this style of experiment attempts to bridge the gap between field and laboratory experiments, by providing much of the control of a laboratory experiment with some of the realism of a field experiment.

Behavioural observations are frequently applied to natural contexts to document behaviours such as foraging (e.g., *Polistes dominulus*: Brown et al. unpubl) or mate-choice (e.g., *Blattella germanica*: Lihoreau et al. 2007). Observing the time spent performing different behaviours can suggest what factors are limiting to the reproductive success of an organism. In this way, behavioural observations may provide insight into the adaptive value of different behaviours. They can also be used to study ontogeny by observing how behavioural patterns change over the lifespan of individual animals. But while behavioural observations carried out in laboratories can add significantly to the range of observable behaviour, the behaviours may differ from those observed in the wild. Animals supplied *ad libitum* food, for example, may engage more frequently in energetically costly behaviours (Kotiaho 2001) and spend less time foraging.

A laboratory invertebrate nesting colony is an example of a natural context that can be manipulated by removing or adding individuals or brood (e.g., *Polistes fuscatus*: Reeve & Nonacs 1992) or altering the nest itself (e.g., ant *Temnothorax curvispinosis*: Pratt 2005). This then allows for detailed manipulations of one factor of the social environment or microhabitat of the animals and can be used to observe habitat selection (Pratt 2005), changes in the dominance hierarchy or changes in interactions among animals (e.g., Nonacs *et al*. 2004).

However, nests kept within a laboratory setting will experience a different environment from those in a field setting and animals may respond differently to nest manipulations in the presence of abundant food and the absence of predators and conspecifics. Laboratory nest manipulations therefore, can provide a first step for testing hypotheses but benefit from being supplemented by observations and complementary experiments carried out in a field setting.

Finally, in a contrived context experiment, the researcher is neither trying to replicate a natural environment nor make the environment as neutral as possible. Instead the researcher provides an artificial environment with novel cues that do not resemble the natural environment. Contrived context experiments can be excellent for determining the factors of the environment that have the largest impact on particular behaviours. For example, an organism can be introduced to an entirely novel environment where their reactions can be used to provide information about their environmental perceptual processing. Novel environment experiments are frequently used to study foraging behaviour (e.g., honeybees *Apis mellifera ligustica*: Sanderson et al. 2006) as many animals will take food from novel sources. Additionally, they can test the ability of organisms to distinguish among different food sources (e.g., Sanderson et al. 2006), or to secure other resources such as nesting material or nest locations. However, it is difficult to know whether animals perceive and interact with an artificial stimulus (e.g., a honeybee feeder) in the same way they do with a natural stimulus (e.g., a flower).

These studies can be used to determine the mechanisms organisms use to make decisions. They can also be applied to study the ontogeny of decision-making by exposing an organism to novel environments at different points in its life-cycle. The major concern with studies using novel environments is that the environment may be so remote from natural as to make the results inapplicable. Novel stimuli may also leave out important cues, such as scents or polarised light and similar studies should be carried out presenting natural stimuli to determine if the same responses are observed.

2.4 Conclusions

In this review, we have detailed select questions of behavioural ecology research to which extensive laboratory methods have been applied, to understand the evolutionary and social context of animal behaviour in vertebrates and invertebrates. Different laboratory contexts have their advantages and disadvantages and none of them are complete alone. Laboratory experiments can be greatly informative for explaining the behavioural patterns observed in the field and can provide extensive information on details of behaviours that cannot be tracked in other ways. Yet, despite their experimental and ontogenetic appeal, such laboratory experiments cannot stand alone. In general, laboratory experiments have more

control but less realism than field experiments and therefore the goal of the laboratory should alternatively be to better understand animal behaviour in the wild. Many of the best experimental progressions of behavioural research take work carried out in the field and elaborate on it in the laboratory or vice versa. Our literature review suggests that future studies should focus on combining both field and laboratory based data to increase theoretical appeal and experimental validity.

3

The strength of species recognition in captive female zebra finches (Taeniopygia guttata): a comparison across estrildid heterospecifics

3.1 Introduction

Species recognition is an essential aspect of animal communication. The ability to recognise conspecifics is paramount for successful sexual reproduction (Irwin & Price 1999) and forgoes the costly production of hybrids that are often infertile or of lower fitness (Coyne & Orr 1989; Hagen 1990; Tegelström & Gelter 1990). Zebra finches are a sexually dimorphic, socially monogamous model system for species recognition research. To date, laboratory studies have assessed female zebra finches' discrimination between male conspecifics of varying traits. These studies include female choice with respect to conspecific male beak colour (Burley & Coopersmith 1987; Collins et al. 1994), song variations (Neubauer 1999; Lauay et al. 2004), and symmetrical chest plumage (Swaddle & Cuthill 1994a) and between conspecifics and heterospecifics using live males of the domesticated Bengalese finch (*Lonchura striata* vars. *domestica)* (Sonnemann & Sjölander 1977; ten Cate & Mug 1984; Brazas & Shimizu 2002), heterosubspecific males of the Timor zebra finch (*T. g. guttata*) (Clayton 1990a, b, c), and song playbacks of phylogeographically distant heterospecifics (e.g., canary, *Serinus canaria* [Lauay et al. 2004]). These studies indicate that females consistently respond more towards conspecific males of a perceivably higher quality and will preferentially select a conspecific over a heterospecific stimulus. However, such preferences can be altered via experimentally manipulated sexual imprinting or learning (Clayton 1990b, c; Riebel 2003a; Witte & Sawka 2003; ten Cate et al. 2006).

Although captive female zebra finches consistently discriminate against domesticated Bengalese finch male heterospecifics, to date there is no research that investigates conspecific vs. heterospecific discrimination behaviour in the presence of a wide-range of geographically diverse and phylogenetically closely or distantly related estrildid finch species. Phylogeographic theory predicts that discrimination against heterospecifics may be influenced by species distribution and phylogenetic history (Coyne & Orr 1989; de Kort & ten Cate 2001) where related species in sympatry may require enhanced discriminatory abilities to avoid costly hybridisation (Dobzhansky 1940; Kirkpatrick & Ravignè 2002). I suggest that species discrimination and mate preference behaviours in captive female zebra finches, raised with exposure to conspecifics only, might vary, when they are presented with a diversity of heterospecifics with which zebra finches have evolved to coexist with in their natural range or that are naturally occurring allopatric species (Kirkpatrick & Ravignè 2002; Price 2008). In my research, I set out to examine the question of

(1) *whether captive female zebra finches recognise and show consistent spatial preference for the conspecific male when faced with a choice of phylogeographically closely or distantly related heterospecific choices in comparison to the frequently used domesticated Bengalese finch stimulus* (Zann 1996).

A fundamental factor relevant for mate selection studies is male behaviour. Previous experiments with zebra finches indicated an interaction between the behaviour of the subject female and that of the stimulus male(s) (reviewed in Collins & ten Cate 1996; Forstmeier 2004, 2007; Holveck & Riebel 2007; Rutstein et al. 2007). Song production and other sexual behaviours, such as bill-wiping, hopping, pivoting, and tail-twisting (Caryl 1976; Zann 1996; Galoch & Bischof 2007), may all aid in attracting the female and enabling her to recognise conspecific males (Vos 1995). Additionally, although female zebra finches do not sing, they do call (Riebel 2003b) and will produce reciprocal courtship behaviours (Zann 1996). I therefore designed my study

(2) *to assess whether captive female zebra finches displayed more calling and courtship behaviours towards the conspecific male to indicate a sexual preference when faced with a choice of phylogeographically closely or distantly related heterospecific choices,* and asked (3) *whether these behaviours were related to the behaviours displayed by the presented male stimulus individuals.*

My study aimed to provide answers to these questions regarding female mate preference in captive-bred zebra finches by using an experimentally novel range of estrildid heterospecific stimuli and comparing the results with data obtained using the same experimental protocol with the heterospecific stimulus of previous studies, the domesticated Bengalese finch.

3.2 Methods

3.2.1 Study subjects

Finches from the family Estrildidae are small passerines that occur naturally across Africa, Arabia, se. Asia, the Pacific Islands, and Australia (Sorenson & Payne 2001; Higgins et al. 2006). The family is composed of 135-140 species of which 20, including the zebra finch are endemic to Australia and occur in partial sympatry, often found in mixed feeding flocks (Zann 1996). Aviary interactions show zebra finches hybridise with multiple estrildid species and the Timor subspecies, of which the latter is the only fertile hybrid produced (Roberts 1988; Clayton 1990c), but the frequency of hybridisation in the wild is unknown (Zann 1996).

For my experiments, I selected five captive-bred species from the estrildid family to be used as heterospecific stimulus males; these included the red-billed firefinch (*Lagonosticta senegala*), orange-breasted waxbill (*Amandava subflava*), plumhead finch (*Neochmia modesta*), red-faced parrot finch (*Erythrura psittacea*), and Bengalese (society) finch. These species represent a broad phylogenetic sampling of estrildid lineages (Sorenson et al. 2004) with respect to the zebra finch (Fig. 3.1) and some will hybridise with zebra finches in captivity (Table 3.1). Because my work was conducted in New Zealand where wild bird importation is prohibited, I specifically acknowledge the limitation in my work (Rutstein et al. 2007) that all finches used were from captive stock that had been bred locally in mixed species aviaries for the heterospecifics and single species aviaries for the zebra finches.

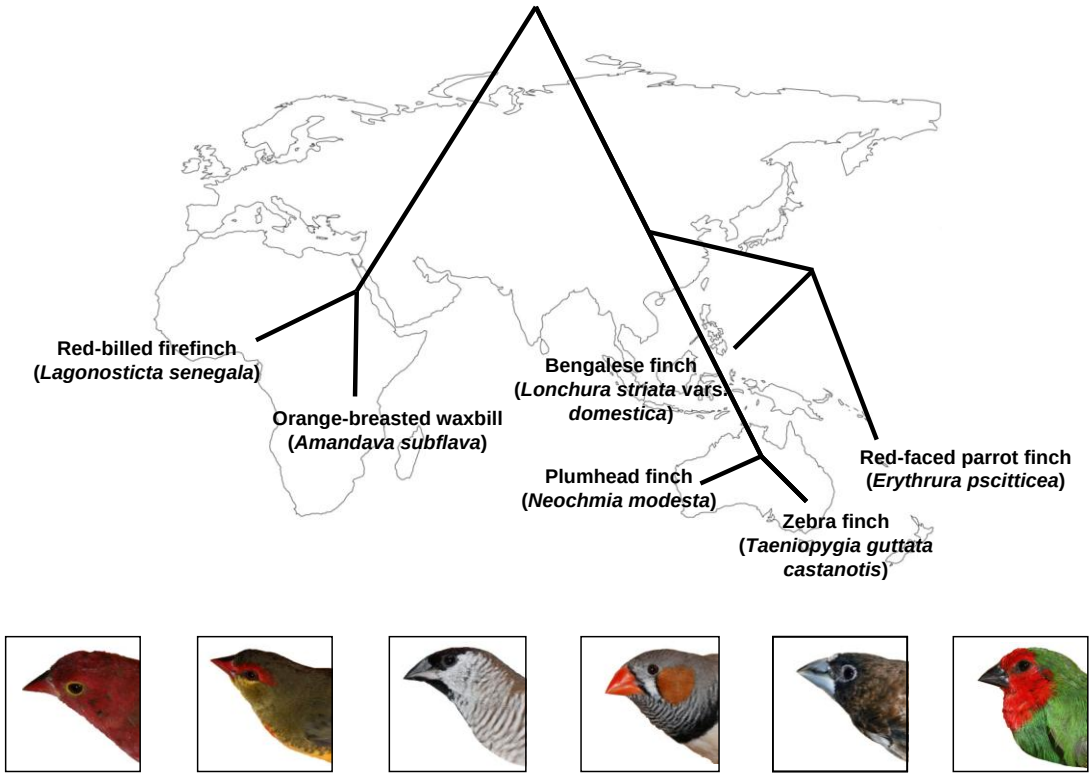


Figure 3.1: The phylogeographic relationship between the estrildid stimuli used. Images from left to right show red-billed firefinch, orange-breasted waxbill, plumhead finch, zebra finch, Bengalese finch and red-faced parrot finch (Photos A. Campbell).

Table 3.1: The average length (cm), weight (g), country of origin and feather and bill colouration of the experimental taxa used (Eisner 1960; Harman & Vriends 1978; Coleman & Whittall 1988; Roberts 1988; Zann 1996; Higgins et al. 2006).

Taxon	**Length (cm)**	**Weight (grams)**	**Country of origin**	**Plumage colouration**	**Hybridisation with zebra finches**
Zebra finch	10.5	12	Australia	*Feathers*: grey, chestnut, black, white *Bill*: red	-
Red-billed firefinch	10	9	Africa	*Feathers*: brown, red, white *Bill*: red	Infertile hybrids
Orange-breasted waxbill	9	7.5	Africa	*Feathers*: orange, red, green-brown *Bill*: red, black	No known hybrids
Plumhead finch	13	12.5	Australia	*Feathers*: olive-brown, white, black, crimson *Bill*: almost black	Infertile hybrids
Red-faced parrot finch	12.5	14	New Caledonia	*Feathers*: green, red *Bill*: black	No known hybrids
Bengalese finch	14.5	12	Domesticated from a Southern Asian finch	*Feathers*: brown, white *Bill*: dark brown	Infertile hybrids

Two unfamiliar, mated males of each of the five heterospecific taxa were used as stimulus birds, each matched with a different wild-type, unfamiliar, unmated zebra finch male (n = 10 different sets of a conspecific and a heterospecific stimulus male). The limitation of working in New Zealand also resulted in my heterospecific stimuli all being mated males, sold in pairs at the time of purchase, while the zebra finch stimuli were unmated males from the University's aviaries. The University's aviaries were 4.0m x 2.0m x 2.5m and housed approximately 20 breeding pairs (individuals 1-3 years old) and any offspring produced. Offspring from these aviaries were removed after reaching sexual maturity (~100 days old) for experimental use as either subjects or stimuli in this chapter and Chapters 4, 5 and 6. All birds used for experimentation throughout this dissertation were less than 2 years old and were used solely for one experiment each (with the exception of female subjects who were used for both Chapters 5 and 6).

To assess the possible effect of the mating status of male heterospecific vs. conspecific stimuli, I obtained additional data from female preference trials between an unmated, unfamiliar male zebra finch and an unmated, unfamiliar chocolate-morph Bengalese finch male. Paired t-tests revealed no significant difference in the spatial responses of the 8

unmated wild-type (n = 5) and fawn (n = 3) zebra finch female test subjects in comparison to the trials with mated Bengalese finch male stimuli (t_7 = 0.097, P = 0.93), and there were no statistical differences in the spatial responses of the female colour morphs within the unmated trials (t_7 = 1.78, P = 0.22). The spatial proximity data in response to the unmated and mated Bengalese finches are shown separately in the figures and no behavioural data were analysed for these unmated trials.

The test subjects for trials using mated heterospecifics were 12 wild-type (n = 7), and fawn (n = 5), unmated captive-bred zebra finch females. There was no statistical difference in the spatial proximity responses towards conspecific males by the two female colour morphs (t_{11} = 1.23, P = 0.28) and so data were combined across morphs.

All birds were colour-banded (blue, black, green or purple) on one leg (except for one female subject), were sexually mature (Zann 1996), and naïve to experimentation.

3.2.2 Housing

Subjects were housed in single sex groups (cage sizes: length 1 m, width 0.6 m, height 1.8 m), in a controlled indoor environment, 35-65% daily humidity, 21 $\pm$ 3 ^{o}C, 16/8 hrs light/dark cycle, including a compact Arcadia^{TM} fluorescent bird lamp 2.4 %UVB and 12 %UVA, on the University of Auckland, City Campus, with all stimulus males held in a separate location from the test subject females (cage sizes: 0.50 x 0.50 x 0.65). All heterospecific stimulus males were housed with their female mate to minimise stress on the birds and all heterospecific pairs were in physical but not acoustic or visual isolation from each other. The female test subjects were visually but not acoustically isolated from 8 breeding zebra finch pairs. Although female subjects were housed in acoustic contact with conspecific song but not heterospecific song, suggesting they might be primed to select the conspecific, this scenario best matches a natural situation where the female would live in a conspecific colony but might need to make a species discrimination decision when flocks become mixed (e.g., during feeding, Zann 1996).

All birds were fed *ad libitum* a commercial finch dry-seed mix **(**Animates^{TM,} Animates LTD, Auckland, New Zealand**)** with fresh water provided daily and spray millet, fresh cucumber, corn, and boiled hen's egg given weekly. Calcium, iodine and grit supplements were also

provided. All birds were monitored on a daily basis and were placed in private aviaries at the completion of the experimentation. Housing and experimental protocols were approved by the University of Auckland Animal Ethics Committee.

3.2.3 Experimental set-up

Experimental trials were conducted on the university campus during January, May-August and December, 2006 in a room fluorescently lit with ambient temperature of 20 $\pm$ 2 ^{o}C. All trials (duration = 10 min) were run daily from 11am local time onwards. Each female subject was placed in the testing cage overnight prior to her set of trials to allow at least 16 hrs to become accustomed to the new surroundings**.** A week prior to the experimental trials each stimulus male was placed in the flanking cages alone (females absent) for one day to habituate. Upon re-entering the cages for each trial they would appear to remember the layout and immediately adapt to the new surroundings (DLMC, pers. obs.).

3.2.4 Testing apparatus

The testing apparatus consisted of three cages, two larger male stimulus cages, A and B (length 1 m, width 0.6 m, height 1.8 m), placed flanking (0.25 m between flanking and central cages) a smaller female subject cage (0.75 m x 0.45 m x 0.45 m) positioned on a bench 0.8 m off the ground (Fig. 3.2). The subject cage was divided into three equal zones, where the centre zone being equidistant from both stimuli was classified as neutral and the outer zones classified as preference or discrimination (Fig. 3.2). The male stimuli cages were also divided into three equal zones although for the later analysis only the behaviours that occurred in the zone closest to the female subject cage were analysed.

Although subject and stimulus males were all in acoustic and visual contact with each other, implying that stimulus interactions and responses to subject/stimulus interactions may have influenced the outcome of my trials (Vignal et al. 2004), this design also best matches natural interactions and was successfully used in many previous trials on species recognition with zebra finches and other taxa (Wynn & Price 1993; Hauber et al. 2000; Doucet et al. 2004). All cages could be visually but not acoustically isolated by dark fabric screens when necessary. Food and water were placed in the neutral zone (Fig. 3.2) in each cage. The entire

setup was located behind a fabric screen positioned 1.3 m from the three cages. Mesh-covered holes permitted the observer to watch all trials unseen.

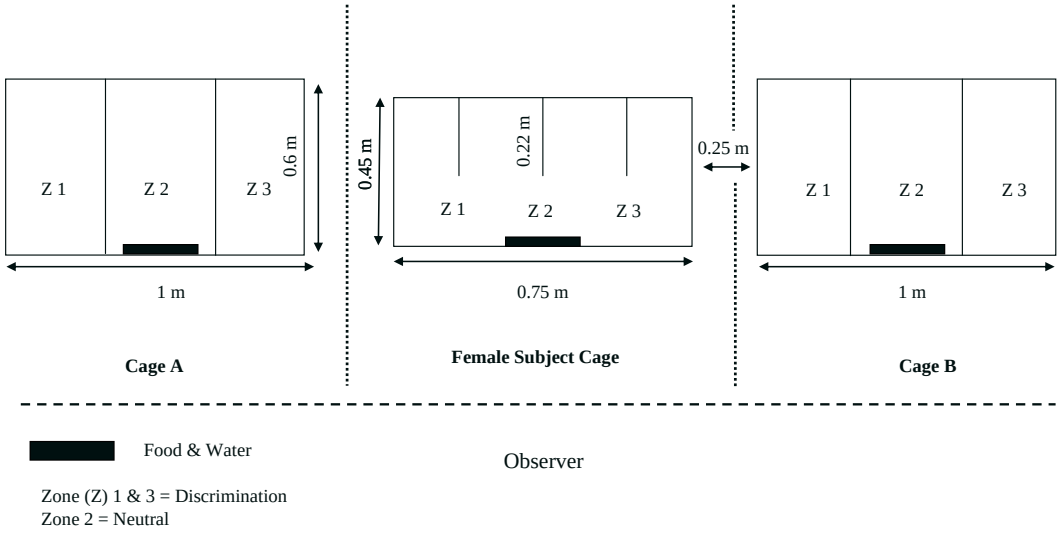


Figure 3.2: A representation of the test apparatus as viewed from above showing the central female subject cage and two flanking stimulus male cages (A & B). The division zones (Z1 [discrimination], Z2 [neutral] & Z3 [discrimination]) are indicated and placement of food and water in the neutral zone depicted. Dotted lines between cages represent the screens to visually isolate the birds and the dashed line represents the screen behind which the observer was positioned.

3.2.5 Experimental procedure

Behavioural choices or discrimination of female zebra finches when presented with conspecific and heterospecific stimulus males were quantified through spatial associations and behavioural displays as procedurally diagrammed in Figure 3.3. A set of stimulus males (one zebra finch and one heterospecific) was introduced into the flanking cages (A and B) and a series of behavioural observation trials was immediately begun where the location (Zone 1, 2 or 3: Fig. 3.2) and behaviours (Caryl 1976; Zann 1996) of each of the three birds were recorded using a scan-sampling design every 10 s.

The procedure of a 4-trial sequence as depicted (Fig. 3.3) was repeated for a single female subject a maximum of 3 times in a single day with 3 different conspecific-heterospecific stimulus pairs. Responses to all 5 heterospecific taxa were tested across two separate 4-trial sequences using two different males. Therefore, in total, the full set of comparative data were collected over 8 trials for each of the 12 female zebra finch test subjects with respect to each

of the 5 conspecific-heterospecific male stimulus pairs, summed to 480 trials. To avoid female biases, the presentation order of stimulus males, testing order of females and pairs of conspecific-heterospecific stimulus males were randomly varied and initial presentation placement of males to Cages A or B was counterbalanced. Due to a delay in obtaining a second male stimulus individual of a red-faced parrot finch, the second set of trials for this species was run in December, 2006 using the above testing protocols and the same female zebra finch subjects.

Data were analysed to provide the proportion of total 'discrimination time' (combined time spent in zones 1 and 3) in each trial that the female spent with the conspecific or heterospecific for each heterospecific stimulus. The data were also analysed for the proportion of total time for each trial that the female subject spent calling and displaying courtship behaviours towards the conspecific and heterospecific stimulus males and the proportion of total time for each trial that the conspecific and heterospecific males spent calling and displaying courtship behaviours (including both Directed [towards the female] and Undirected singing [Zann 1996]) while in the zone of closest proximity to the females' cage. All behaviours within sets of trials were measured by the same observer (DLMC or RCS) and averaged for each individual female subject in relation to each heterospecific stimulus taxon. The observer was not blind to the experimental aims. Those trials where the female remained in the neutral zone for the entirety of the trial and did not discriminate were excluded from any analyses (n = 4).

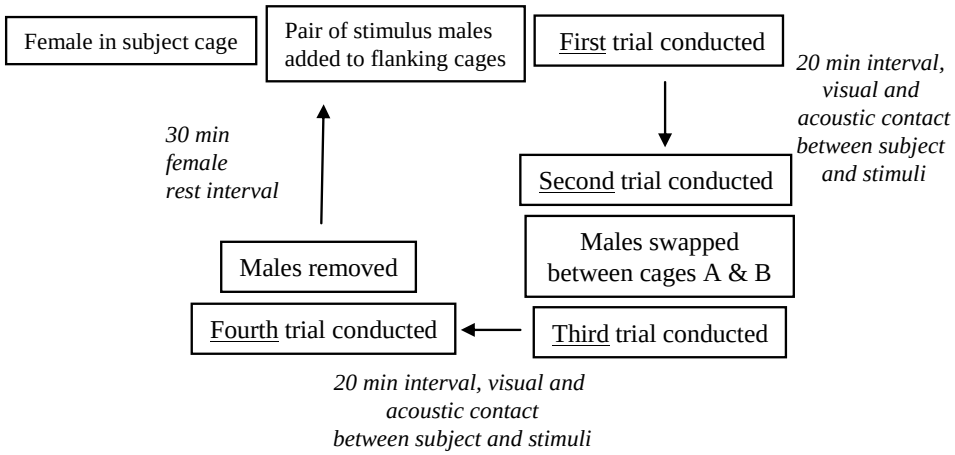


Figure 3.3: A schematic of the experimental trial procedure for testing each female subject with a pair of stimulus males.

3.2.6 Statistical analyses

All proportional data were initially log-transformed ($\log_{10}$ (X + 1)), followed by parametric tests to assess significance ($\alpha < 0.05$). Statview 5.0.1 and JMP 7 (Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA) were used for statistical analyses. All tests are two-tailed. Generalised Linear Mixed Model (GLMM) analyses were applied to the log-transformed proportions of total 'discrimination time' in the proximity of the conspecific male stimulus with female identity included as a random effect. GLMM with female identity as a random effect, was also used to study differences in the calling and courtship behaviours displayed by the subject females towards the conspecifics and heterospecifics, in relation to each presented heterospecific, and displayed by the conspecific and heterospecific stimulus males. Student's t-tests were applied the least squares means estimates to assess *post hoc* differences and linear regressions were employed to explore the relationship between subject and stimuli behaviour for conspecifics and heterospecifics across each taxon.

For illustrations non-transformed raw proportional values are presented in the figures.

3.3 Results

Captive female zebra finches showed significant spatial association with conspecific males ($F_{1,107} = 196.42$, $P < 0.0001$) but contrary to expectations of greater discrimination against the biologically, phylogeographically, relevant heterospecifics (Kirkpatrick & Ravignè 2002; Price 2008), total 'discrimination time' that females spent in the proximity of the conspecific against each of the five heterospecific stimulus taxa showed no statistical difference with regards to the identity of the heterospecific stimuli ($F_{4,44} = 0.12$, $P = 0.97$) (Fig. 3.4). The raw proportional data for spatial responses towards the unmated Bengalese finch stimulus male are included in the figure.

Similar to patterns of spatial discrimination, and, as expected, I found that the female zebra finches directed significantly more calling and courtship behaviours towards the conspecific in the presence of all heterospecific stimuli combined ($F_{1,103} = 62.53$, $P < 0.0001$) (Fig. 3.5). The proportions of calling and courtship behaviours were consistent when directed towards the conspecific ($F_{4,44} = 0.48$, $P = 0.75$) but there was an effect of taxon in proportions directed towards the heterospecifics ($F_{4,44} = 2.75$, $P = 0.04$). The behaviours towards the

Bengalese finches significantly differed from only two of the other presented taxa (plumhead finch and red-billed firefinch) (Fig. 3.5).

As expected, there were also significantly more calling and courtship behaviours displayed by the conspecific males over the heterospecific males across all taxa ($F_{1,103} = 92.12$, $P < 0.0001$) (Fig. 3.6). Similar to female behaviours, these were consistent across the conspecific males in relation to the presented heterospecific stimulus taxa ($F_{4,44} = 1.91$, $P = 0.13$) but significantly varied between the heterospecifics ($F_{4,44} = 10.15$, $P < 0.0001$), with the Bengalese finches displaying significantly more than all other presented heterospecifics (Fig. 3.6).

In contrast to my expectation, in the presence of each heterospecific stimulus taxon, there was only one significant interaction between the female subjects' behaviour towards the conspecifics in relation to the conspecific males' behaviour, (conspecific/Bengalese finches $R^2 = 0.37$, $P = 0.034$), all other interactions were not significant ($R^2 \leq 0.19$, $P \geq 0.16$). Only one significant interaction between the female subjects' behaviour towards the heterospecifics in relations to the heterospecific males' behaviour was detected (orange-breasted waxbills $R^2 = 0.34$, $P = 0.05$), all other interactions were not significant ($R^2 \leq 0.031$, $P \geq 0.56$).

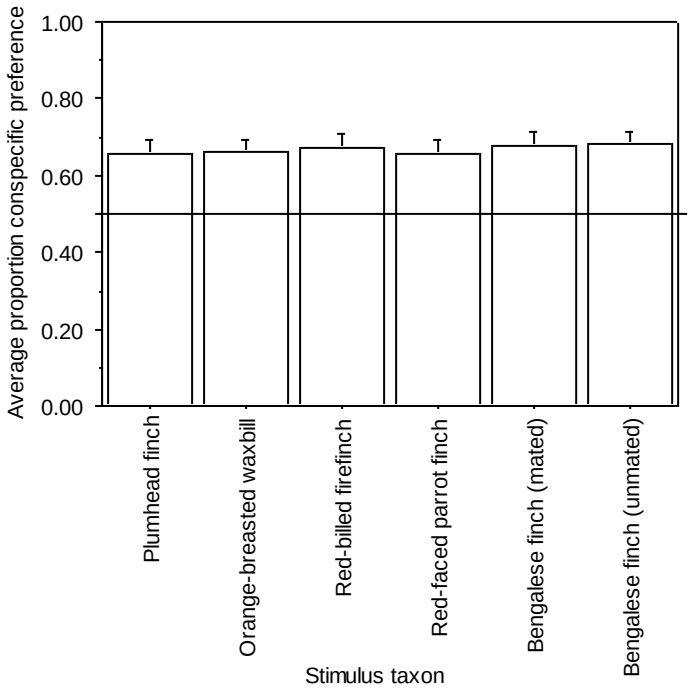


Figure 3.4: Proportions of total 'discrimination time' the female zebra finch preferred the conspecific in the presence of the five heterospecific stimulus taxa (data shown for both mated and unmated Bengalese finch stimuli). Mean of the raw proportional values + SE depicted and the solid line indicates random choice of 50%.

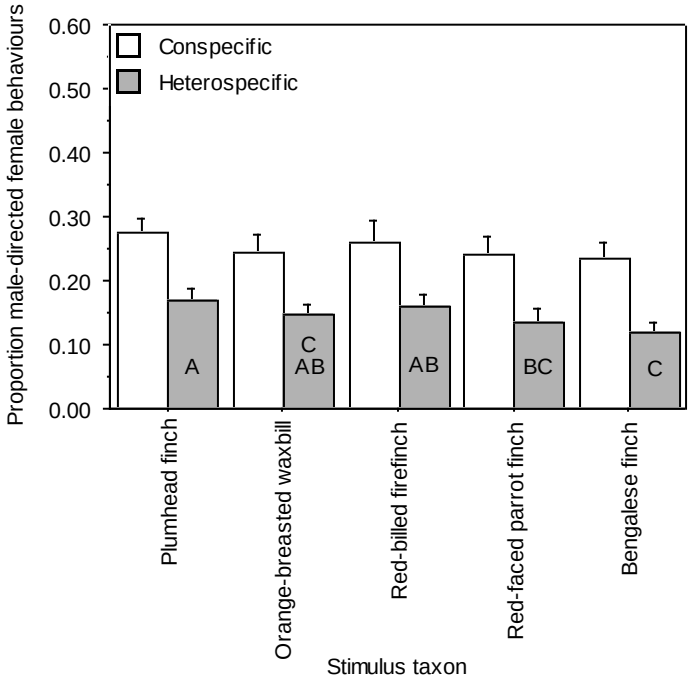


Figure 3.5: Proportions of female calling and courtship behaviours directed towards the male conspecifics and heterospecifics for each presented heterospecific stimulus taxa. Mean of the raw proportional values + SE depicted and heterospecifics without the same letter showed significant *post hoc* differences. All pairwise t-test comparisons between conspecific and heterospecific-directed behaviours for each stimulus taxon are significant.

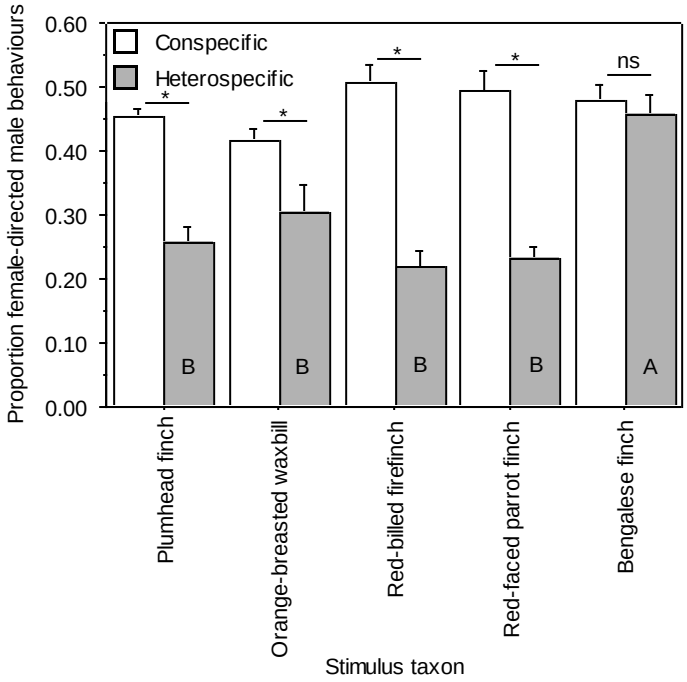


Figure 3.6: Proportions of male conspecific and heterospecific calling and courtship behaviours towards the female zebra finches for each heterospecific stimulus taxon. Mean of the raw proportional values + SE depicted, heterospecifics without the same letter showed significant *post hoc* differences and *asterisk* indicates significant pairwise t-test comparisons between conspecifics and heterospecifics for each stimulus taxon.

3.4 Discussion

The results of my experiments revealed several consistent outcomes regarding spatial association with and behavioural responses towards conspecific males by captive female zebra finches in the presence of a broad range of heterospecific male stimuli. These results matched previous empirical research on species discrimination in captive zebra finches, but gave little support for subject and stimuli behavioural interactions and were varying in regards to theoretical expectations based on phylogeographic history (Kirkpatrick & Ravignè 2002; Price 2008).

Specifically, female zebra finches across these trials, in a choice scenario, showed a consistent spatial and behavioural preference for conspecific stimuli irrespective of the identity of heterospecific male stimuli. This is in line with the results of other studies that have looked at hetero(sub)specific choice using live Timor zebra finches (Clayton 1990a) and domesticated Bengalese finch males versus Australian zebra finch males (Brazas & Shimizu 2002). However, despite the consistency in behavioural responses towards the conspecifics there were species differences in behaviours directed towards the heterospecifics, although not all novel, biologically relevant heterospecifics elicited different behavioural responses from the commonly used Bengalese finches as I had predicted (Kirkpatrick & Ravignè 2002; Price 2008).

The wide-range of experimentally novel visual and acoustic heterospecific stimulus cues of live males that were presented in the choice test to female zebra finches resulted in the females responding to these cues in different ways, showing varying levels of discrimination. The non-negligible quantities of male heterospecific-directed behaviours by the females may be due to possible similarity in male finch behaviours across multiple species (Morris 1958) or female curiosity for novel species, particularly given that these females had no prior exposure to related heterospecifics. But despite the variation in these heterospecific-directed behaviours the female showed consistent strength of preference for the conspecific which is in agreement with the concept of own-species bias (Lauay et al. 2004) and may be based on some unique signal that defines the conspecific. Such critical conspecific recognition cues (i.e., passwords) have been suggested to trigger conspecific identification in the brood parasitic brown-headed cowbird (*Molothrus ater*) as shown by Hauber et al. (2001).

Comparisons of specific vocal and visual cues in future studies should help define the key signals used for correct discrimination.

The conspecific stimulus males were found to consistently respond towards the female subject more than the heterospecific stimulus males who displayed varying levels of calling and courtship behaviours while in close proximity to the female subject. These behavioural responses were expected given that the discrimination process is dynamic between all individuals involved in a simultaneous choice paradigm (Collins 1994) but I detected only two (out of 10 interaction combinations) statistically significant interactions between the behaviours of the subject and stimulus individuals. Previous studies using song measures only have shown females to be attracted to and to preferentially select those males that display at a higher rate (Sonnemann & Sjölander 1977; Collins et al. 1994; ten Cate & Mug 1984). In turn these males are further motivated to display by the presence of a female, suggesting the behavioural presentations are interdependent (Collins 1994). Recent experimental evidence suggests song rate itself is not the primary influencing trait in female choice (Forstmeier 2007; Rutstein et al. 2007), but it is the song structure that is important for female decisions (Holveck & Riebel 2007) which may account for my experimental findings. Although I measured a broad range of calling and courtship behaviours performed by the male stimuli, including Directed song, I still found little evidence of a statistical relationship which indicates the stability of the females' discrimination decision that is not strongly influenced by the courting males (Forstmeier 2004). I do suggest, however, that the conspecific males' behaviour likely played a part in aiding the recognition by the female (Vos 1995).

Despite these findings, it remains to be examined whether wild-caught zebra finch females (Zann 1996; Rutstein et al. 2007), which are typically raised by parents and amongst siblings but which also interact with sympatric estrildids and other heterospecifics, or captive-bred birds raised in a mixed-species aviary, would have reacted to the stimuli presented in a different manner to my captive-bred, conspecific-socialised birds. Additionally, although my measures included some sexual behaviours of subjects and used subject and stimulus individuals of opposite sexes, I cannot reject the notion that the preferences I observed were purely social (i.e., related to foraging, roosting, or predator-avoidance) and not sexual or reproductive in motivation. Zebra finches are by nature highly social birds and will associate with conspecifics of either sex in a spatial paradigm (Vos 1995). The behaviours I measured

were not entirely exclusive to sexual or mate choice contexts and further free-flight pair bonding tests would be needed to assess and validate the motivation behind the reported spatial preferences (ten Cate 1985).

My study has shown that when presented with a range of male stimuli in a laboratory-based choice scenario, captive-bred female zebra finches consistently prefer to spatially associate with and behaviourally respond to conspecific over heterospecific males and that response is little influenced by stimulus behaviour. The results indicate accurate species recognition irrespective of heterospecific identity. My data, to some degree, validate extensive previous work on the social, acoustic and developmental basis of species recognition in captive zebra finches as a model system (Zann 1996; Göth & Hauber 2004) and provide a basis for future research on mate preference in zebra finches and the cues and mechanisms involved during reliable species identification.

4

Spatial and behavioural measures of social discrimination by captive male zebra finches: implications of sexual and species differences for recognition research

4.1 Introduction

The identification of conspecifics is essential for successful breeding in sexually reproducing species, which include all birds (Irwin & Price 1999). Accurate mate selection prevents the production of costly hybrids (Hagen 1990; Sætre et al. 1997; Tegelström & Gelter 1990; but see Grant & Grant 1997) and is critical in monogamous systems where both sexes invest and cooperate in parental care necessary for offspring survival (Burley 1977; Bart & Tornes 1989). Conspecific identification may occur via the accurate recognition of species-specific visual and/or vocal cues (Ratcliffe & Grant 1983; Price 1998; Irwin & Price 1999; Göth & Hauber 2004) and typically involves a comparison and preference decision made between multiple individuals (Ryan & Rand, 1993; Ryan et al. 2007). A model species for investigating species recognition systems is the zebra finch (*Taeniopygia guttata castanotis*), a small, sexually dimorphic, biparental Australian passerine of the estrildid family of finches (Sorenson et al. 2004), because it is well-suited to captive studies with its year-round breeding and short developmental time (Zann, 1996 but see Rutstein et al. 2007).

There has been much research exploring the traits involved in captive-bred female zebra finches' preference for male conspecifics, including males' bill colour (Burley & Coopersmith 1987; Collins & ten Cate 1996), leg band colour (Burley et al. 1982), leg band symmetry (Swaddle & Cuthill 1994a), chest band symmetry (Swaddle & Cuthill 1994b) and song rate (Collins 1994; Collins & ten Cate 1996). There is also evidence that female zebra finches prefer males of their own subspecies, compared to male heterospecifics using domesticated Bengalese finch males (*Lonchura striata* vars. *domestica)* (Sonnemann & Sjölander 1977; ten Cate & Mug 1984; Brazas & Shimizu 2002) or heterosubspecific males of the Timor zebra finch (*T. g. guttata*: Clayton 1990a, b, c).

In contrast, there have been few investigations of mate preference and species recognition in male zebra finches (Vos et al. 1993). As male zebra finches invest heavily in incubating and provisioning for young through biparental care, they are also expected to exhibit preferential mate selection criteria (Wynn & Price 1993). There is evidence to show that captive-bred male zebra finches have preferences for certain conspecific females with regards to variation in leg-band or beak colours (Burley et al. 1982; Burley 1985, 1986; Burley & Coopersmith 1987), weight (Ikebuchi & Okanoya 2006), perceived fecundity (Monaghan et al. 1996; Jones et al. 2001), and condition (Wynn & Price 1993). In addition, species discrimination

tests demonstrate that male zebra finches prefer a female conspecific over females of a domesticated heterospecific estrildid, the Bengalese finch (ten Cate 1982, 1985; Clayton 1987a; Galoch & Bischof 2007). Finally, free-flight aviary tests show positive assortative mating (Böhner et al. 1984) between males and females within the two subspecies of zebra finch (*T. g. castanotis* and *T. g. guttata*).

Currently no research is published on the conspecific preference of zebra finches in the face of phylogeographically relevant heterospecific finches, which include sympatrically distributed and/or phylogenetically close estrildid finches. I recently conducted a series of discrimination experiments using female zebra finches that documented their response to males of various heterospecific estrildid finches (Chapter 3). Related species that live in sympatry are suggested to have a greater evolutionary benefit of species recognition to avoid any costly hybridisation (Kirkpatrick & Ravigné 2002; Price 2008). Therefore, it is predicted that sympatric vs. allopatric heterospecific stimulus taxa might elicit stronger vs. weaker strengths of conspecific preference, respectively. Alternatively, a similar strength of conspecific preference over multiple heterospecifics validates the use of the domesticated Bengalese finch in past and future species recognition studies with captive-bred male zebra finches. To these aims, I collected spatial and behavioural measures of species discrimination by male zebra finches in a simultaneous pairwise social choice paradigm using phylogeographically relevant estrildid finch species, and included the Bengalese finch as a comparison. Specifically, I asked (1) *whether captive-bred male zebra finches displayed consistent spatial proximity and more calling and courtship behaviours towards the conspecific female to indicate a social and sexual preference when faced with a choice of phylogeographically close or distant heterospecific choices*.

As documented in studies of female mate preference, the behaviour of the stimulus birds may have an effect on subject discrimination (ten Cate & Mug 1984; Collins 1994; Collins & ten Cate 1996; Forstmeier 2004). Therefore, the behaviours of the female stimuli in male mate preference trials and their possible impacts on decision-making behaviour are important factors to take into account (Jones et al. 2001), although the causal relationship between proximity and courtship displays may be difficult to determine (Collins 1994). Female zebra finches do not sing, but do call and exhibit courtship behaviours such as bill-wiping, hopping, pivoting and tail quivering to indicate their sexual interest (Morris 1954; Goodwin 1982; Zann 1996), of which tail quivering is also correlated with spatial proximity choice

(Witte 2006). Accordingly, in my experiments I also asked (2) *whether the calling and courtship behaviours of the female stimuli correlated with male zebra finch subjects' behaviours and differed between the conspecific and heterospecific females*.

Sexual selection theory predicts that the female sex will invest more time in their reproductive mating decisions due to a higher initial reproductive cost invested in gametes, even when parental care is equitably shared between socially monogamous pairs (Burley 1977; Andersson 1994; Jennions & Petrie 1997). For hybridisation that occurs in wild populations, the more fastidious female is more likely to be of a rarer species (Wirtz 1999). This suggests that conspecific mate selection is more critically fitness-relevant and, thus, should be more accurate for females (Randler 2002). Accordingly, in a species recognition context, males might be less discriminating than females of the same species (Reeve 1989; Wirtz 1999; Luddem et al. 2004; Tynkkynen 2008). In my previous experiment I assessed captive-bred female zebra finches' conspecific preferences using the same four phylogeographically relevant species and compared these results with my own data on Bengalese finches (Chapter 3). In the present experiments I therefore also asked (3) *whether captive-bred male zebra finches are less discriminating in their conspecific preference than captive-bred female zebra finches when presented with the same set of con- and heterospecific stimuli of the opposite sex.*

Finally, individual subjects' variability in social and sexual behaviours represent biologically and methodologically critical implications for experimental choice paradigms (Forstmeier & Birkhead 2004). I incorporated this level of variation into my analyses by accounting for individual identity using repeated-measures type statistical analyses for questions (1-3) but I also quantified (4) *to what extent do female and male subjects' discrimination behaviours vary between the different heterospecific stimulus taxa*.

4.2 Methods

4.2.1 Study subjects

A total of 32 birds were used for this study. Tested subjects were 12 wild-type unmated captive-bred zebra finch males. Different sets of individual conspecific and heterospecific

females were presented as stimuli. Each of these sets (n = 10) consisted of 1 wild-type, unfamiliar, mated zebra finch female (out of 10 individuals), and 1 (out of two available exemplars each) unfamiliar, mated female of each of the five heterospecific taxa.

The zebra finch is native to Australasia and lives in sympatry with several other estrildid species where they can be found in mixed species feeding flocks (Zann 1996). Although estimates for the rate of hybridisation in the wild are unavailable, aviary interactions have documented infertile hybridisation of the Australian subspecies of zebra finches (*T. g. castanotis*) with several sympatric and allopatric estrildid species (Roberts 1988) and fertile hybridisation with the Timor subspecies of the zebra finch itself (*T. g. guttata*) (Clayton 1990c).

As per my previous experiment, that investigated species discrimination in female zebra finches (Chapter 3), for my current study using male zebra finch subjects I selected the same five captive-bred species from the estrildid family to be used as heterospecific stimuli: red-billed firefinch (*Lagonosticta senegala*), orange-breasted waxbill (*Amandava subflava*), plumhead finch (*Neochmia modesta*), red-faced parrot finch (*Erythrura psittacea*), and Bengalese finch for comparison with prior published work (also: society finch) (Fig. 4.1). In New Zealand all estrildid finches available for experimentation are captive-sourced birds from within a limited range of species because of a long-standing import embargo on wild bird species into this country. Therefore all heterospecific finches (with the exception of the Bengalese finch) were available only in mated pairs as they are not sold individually. For methodological consistency (Vignal et al. 2004), each zebra finch female also came from a mated pair.

All heterospecific birds were purchased from breeders around Auckland, New Zealand, for the experiment and identified with a numbered red leg band. All zebra finches were bred in the University's aviaries and socially exposed to conspecifics only. Zebra finch subjects and stimuli were selected from separate aviaries to ensure unfamiliarity. All test and stimulus birds were sexually mature (Zann 1996), less than two years old and naïve to experimentation.

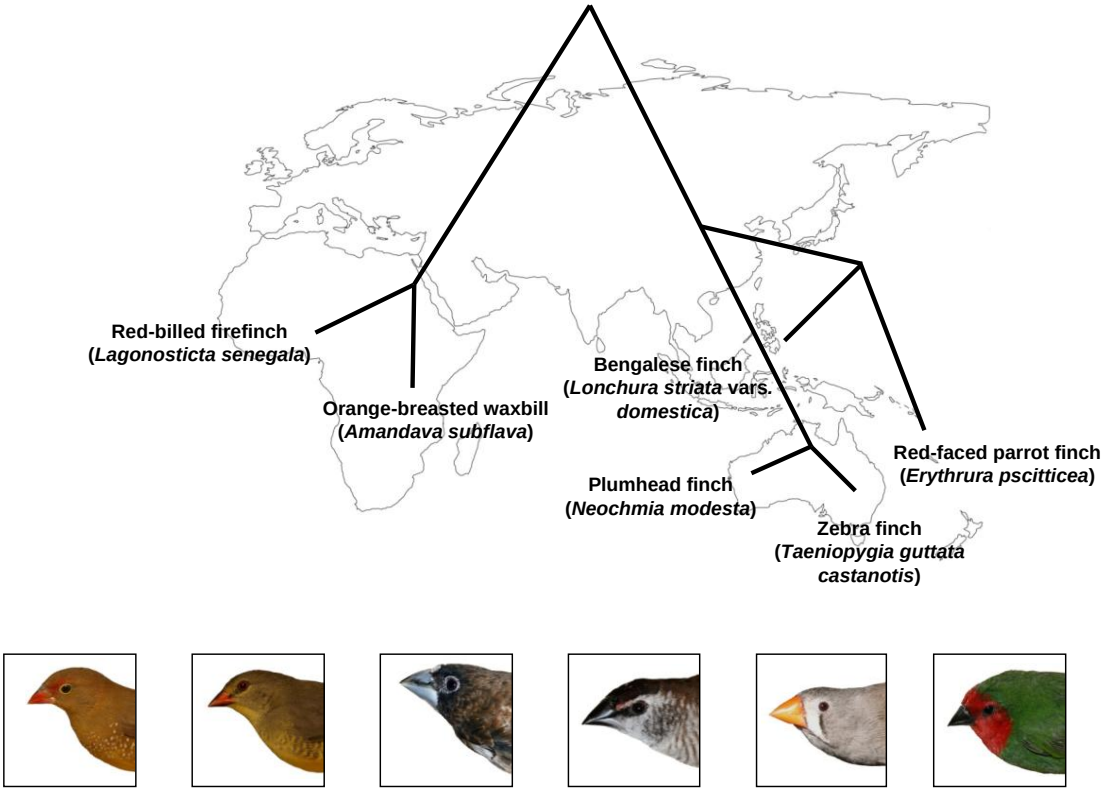


Figure 4.1: A map showing the phylogeographic relationship (Sorenson et al. 2004) between the estrildid stimuli used. Images from left to right show profiles of female red-billed firefinch, orange-breasted waxbill, Bengalese finch, plumhead finch, zebra finch, and red-faced parrot finch (Photos A. Campbell and G. Wheeler).

4.2.2 Housing

Housing and experiments were approved and repeatedly monitored by the University of Auckland Animal Ethics Committee and the university's independent animal welfare officer. Zebra finch subjects and stimuli were housed in a controlled environment on the University of Auckland, City Campus. The heterospecific female stimuli were kept in a remote outdoor aviary until required for experimentation and were then housed (with their mate) in a sound-proof chamber for the duration of the experimental trials only.

Male test subjects were kept in single sex groups in an animal housing cabinet at 20 $\pm$ 2 ^{o}C on a 14/10 hrs fluorescent light/dark cycle (size of cages: 0.50 x 0.50 x 0.65). They were

housed in visual but not acoustic isolation from a group of zebra finch females not used in the experiment. Stimulus conspecific females and their mates were kept separate from the male subjects in a room at 21 $\pm$ 3 ^{o}C with fluorescent lighting provided on a 16/8 hrs light/dark cycle including a compact Arcadia^{TM} fluorescent bird lamp, 2.4 % UVB and 12 % UVA (size of cages: 0.48 x 0.30 x 0.38).

All birds were fed *ad libitum* a commercial finch dry-seed mix (Animates ^{TM}) with fresh water provided daily and spray millet, fresh cucumber, corn, rice, and crushed whole boiled hen's egg given weekly. Supplements of calcium, iodine and grit were also given. All birds were monitored daily and cages were cleaned weekly.

4.2.3 Experimental set-up

Experimental trials were run daily between May and July 2007 beginning from 11am (NZST) onwards and adhered to a standardised schedule across trials. The testing room was fluorescently lit and the temperature averaged 20 $\pm$ 2 ^{o}C. Each male test subject was placed in the test cage overnight prior to its set of trials to allow at least 16 hrs to adjust to the new environment and each stimulus female placed in the flanking cages alone, to habituate for 1 day prior to the experimental trials. The stimulus females immediately adapted to the familiar surroundings upon re-introduction for each trial sequence (DLMC, pers. obs).

4.2.4 Testing apparatus

The testing apparatus consisted of three cages and was identical to that used in Chapter 3 (Fig. 4.2). Two larger female stimulus cages (A and B: length 1.0 m, width 0.6 m, height 1.8 m) were placed flanking (0.25 m separation) a smaller male subject cage (0.75 x 0.45 x 0.45) that was positioned on a bench 0.8 m off the ground (Fig. 4.2). All cages were divided with perches into 3 equal zones (Fig. 4.2) and stimulus females were in visual and acoustic contact with each other and with subject males. This contact design was deemed to best match natural interactions and has been successfully employed in numerous previous studies on species recognition with zebra finches and other taxa (cf. Wynn & Price 1993; Hauber et al. 2000; Hauber 2002; Doucet et al. 2004; Vignal et al. 2004). I do acknowledge that the large size of the stimulus cages specifically employed in my design may have led to some female stimuli being closer to the subject male than others during the trials which in turn may have

significantly influenced the males' choice behaviour. I corrected for this potential effect in my observations and subsequent analyses as later described. All cages could be visually but not acoustically isolated by dark fabric screens when necessary between trials. Food and water were placed in the central zone of each cage. The testing apparatus was located behind a fabric screen at a distance of 1.3 m from the three cages. Mesh holes permitted the observer to watch and record in real time all trials while not interfering with the animals' behaviour.

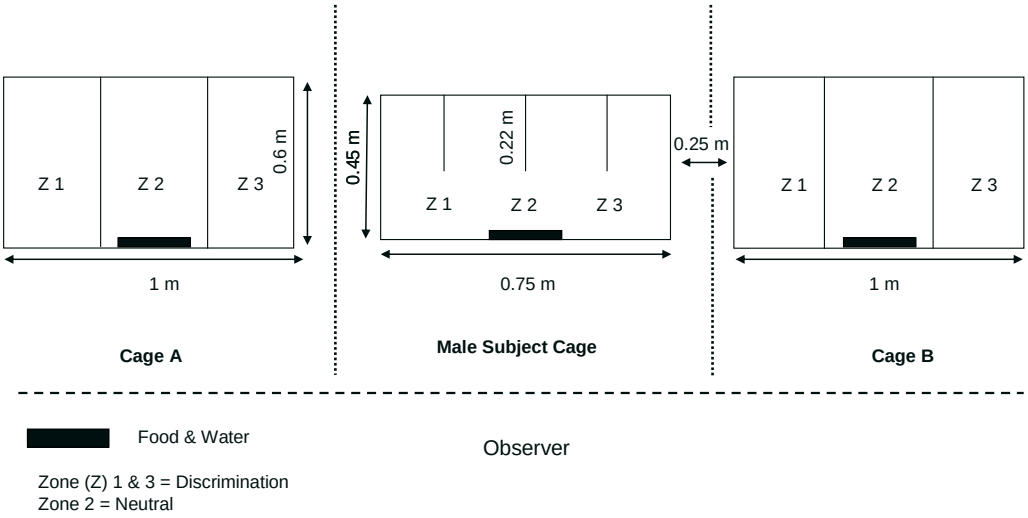


Figure 4.2: A representation of the testing apparatus as viewed from above showing the central male subject cage and two flanking stimulus female cages (A and B). The division zones (Z1 [discrimination], Z2 [neutral] and Z3 [discrimination]) are indicated and placement of food and water in the neutral zone depicted. Dotted lines between cages represent the screens to visually isolate the birds and the dashed line represents the screen behind which the observer was positioned.

4.2.5 Experimental procedure

Behavioural discrimination by male zebra finches when presented with mated conspecific and heterospecific female stimuli was quantified through spatial associations and behavioural responses. The subject cage was divided with three perches into three equal zones, with the central perch/zone being equidistant from both stimuli and classified as neutral. The outer perches/zones were classified as choice or discrimination. The stimulus cages were also divided into three equal zones where the two perches were classed as being in zones 1 and 3 (Fig. 4.2) and the neutral zone included the food/water only. It must be noted that these division zones could not be defined specifically as per the central subject cage zones because

there was not equal stimulation in the outer zones from two flanking cages. Therefore for the later analysis only the behaviours that occurred in the zone proximal to the test cage were analysed. A set of stimulus females was introduced into the flanking cages (one female each in A and B) in an initially random order (see below) and a behavioural observation trial was started. The conspecific and heterospecific female stimuli allowed the assessment of conspecific discrimination in a simultaneous choice context and, thus, enabled the estimation of the relative strength of conspecific preference depending on the presented heterospecific taxon in a sequential design (Wagner 1998).

All trials had a duration of 10 min. The locations of each of the three birds and their behaviours (Morris 1954; Caryl 1976; Goodwin 1982; Zann 1996) were recorded using a scan-sampling design every 10 s. The behaviours included both *directed* and *undirected songs* performed by the males as well as *calling* and additional courtship behaviours (*bill-wiping, hopping, pivoting, tail-twisting* (males only) and *tail quivering* (females only) [Morris 1954; Goodwin 1982; Zann 1996]) that were displayed by the male subjects and female stimuli when in close proximity to each other. Male courtship behaviours quantified for analyses were defined as the above-mentioned behaviours occurring in zones closest to the female stimuli (excludes neutral zone) and when the male was oriented towards the female. These behaviours would most often occur in sequence and were therefore distinguished from similar behaviours (e.g., hopping) occurring in a non-sexual context. All behavioural observations were made by the same observer (DLMC). The sexual displays performed by females of the species used as heterospecific stimuli in general are rarely documented (Zann 1976). Therefore, the same behavioural categories for both conspecific and heterospecific female stimuli were identified and recorded.

After the first trial the birds were left in visual and acoustic contact for 20 min and then a second trial was run. The dark screens between the subject and stimulus cages were lowered, the females' locations swapped between cages A and B, screens raised and a third trial run. The birds were then left again for 20 min and finally a fourth trial was conducted. In summary, a sequence of 4-trials with even sidedness of con-/heterospecific stimulus presentations was run for each male subject before the stimulus females were removed, the male subject was left alone with screens lowered for a 30 min rest interval and another set of stimulus females was introduced. The presentation order of stimulus females and the testing order of males were randomised. Initial presentation placement of females to cages A or B

was counterbalanced and pairs of stimulus females were randomly varied to avoid the effects of possible male cage side bias and possible discrimination bias via avoidance of certain individuals.

In total, data were collected over 8 trials (4 trials for each of the two available heterospecific female individuals, each randomly matched with a different conspecific female) for each of the 12 male zebra finch test subjects with respect to each of the 5 heterospecific versus conspecific female stimulus sets (480 trials in total). There was no difference in male spatial or behavioural responses across trials 1-4 for each heterospecific individual with respect to the 5 heterospecific taxa as assessed in one-way ANOVA analyses (all $P \geq 0.10$). Paired t-tests also revealed no statistical difference in the spatial or behavioural responses of males towards the two different con-/heterospecific sets for each stimulus taxon (all $P \geq 0.13$). Furthermore, there was also no statistical difference in the relative preference for the conspecific between the two different con-/heterospecific pairs for each stimulus taxon (paired t-tests: all $P \geq 0.10$) indicating that the males did not habituate to the consistent presentation of conspecific individuals against varying heterospecific taxa. Therefore male spatial and behavioural responses were averaged across the 8 trials for each subject with respect to the 5 heterospecific taxa.

Data were analysed to provide the proportion of total 'discrimination time' (combined time spent in the proximity zone with either stimulus female) in each trial that the male spent with the conspecific or heterospecific for each heterospecific stimulus taxon. This was used to calculate the average proportion of total observations across the entire data set, collectively for all 12 subjects. Note that this was not a measure of real time (s) (cf. Witte & Caspers 2006) but a proportion of the 10 s samples recorded within the 10 min trial. All data points of trials in which male subjects exhibited a 100 % side bias (i.e., they spent the entire time on one cage side for a particular 4-trial sequence for one presented stimulus pair) were removed from the dataset for the 4-trial sequence in which they exhibited such a bias. This 100 % side bias occurred on 10 occasions (out of the 120 4-trial sequences) and led to a reduction in the total 'discrimination time' subject number from 12 subjects to 11 each for the red-billed firefinch and red-faced parrot finch sets of trials. These subjects were then also removed from the behavioural display datasets as described below. Interestingly, such 100 % side bias did not occur for the female zebra finch subjects in the previous experiments (Chapter 3). The individual 10 min trials in which the male subject remained in the neutral zone for the

entirety of the trial and did not discriminate were also removed from any analyses (n = 3 trials).

The data were also analysed with respect to the combined proportion of the observations for each 10 min trial that the male subject spent performing directed song, calling and additional courtship behaviours when in proximity to the conspecific and heterospecific female stimuli. An average value was calculated for the proportions of total observations in which directed-song only and total combined calling and courtship behaviours (including directed-song) were directed towards the conspecific or heterospecific females for each heterospecific taxon. This was averaged across each subject for each heterospecific across the two trial sets combined. Behavioural data were also analysed for the combined proportion of observations that the female stimuli spent calling and performing courtship behaviours, but only when resident in the zone proximal to the central male subject cage (to control for the large spatial expanse in the stimuli cages), and irrespective of male subjects' spatial proximity. The data were totalled for the average courtship display presented to each male subject by the female conspecific and heterospecific stimuli across all con/heterospecific trial sets combined.

4.3 Results

All proportion data were initially log transformed ($\log_{10}(X + 1)$), followed by parametric and non-parametric tests to assess significance ($\alpha < 0.05$) using Statview 5.0.1 and JMP 7 (Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA) for the analyses. Illustrations of raw data and analyses are presented in figures 4.3 to 4.6.

For an exploratory approach I used a one-sample t-test for each of the five con-/heterospecific stimulus sets on individual subjects' average 'discrimination time' spent with the conspecific female against a random expectation of 50 % (log-transformed: 0.176). Based on previous work documenting directed-song responses of male zebra finches (ten Cate 1985; Clayton 1987a) it was predicted that individual male zebra finches as a group would show consistent spatial association with conspecific over heterospecific female stimuli. But contrary to my expectations, captive male zebra finches showed no consistent spatial association with conspecific females above random expectations in the presence of any of the five different heterospecific stimuli (plumhead finch $t_{11} = 0.79$, $P = 0.44$; orange-breasted

waxbill t_{11} = 0.99, P = 0.34; red-billed firefinch t_{10} = 1.31, P = 0.29; red-faced parrot finch t_{10} = -0.44, P = 0.67; Bengalese finch t_{11} = 0.09, P = 0.93 (Fig. 4.3).

To further evaluate my predictions, I carried out a Generalised Linear Mixed Model (GLMM) analysis on the heterospecific stimulus-taxon averaged 'discrimination time' (5 levels for the heterospecific-taxon effect variable) in the proximity of the conspecific female stimulus with the identity of the male subject included as a random effect (i.e., in these analyses each male subject contributed one data point per heterospecific taxon). It was predicted that males would show increased discrimination against the Australasian heterospecifics in comparison to African finches, including phylogenetically more distant taxa and the domesticated Bengalese finch, because the former are biologically more relevant due to proximal evolutionary histories and geographic distributions with zebra finches. But again, there was no statistical pattern of spatial proximity with the conspecific female stimuli over any of the five heterospecific taxa ($F_{4,42.11}$ = 0.56, P = 0.70) (Fig. 4.3).

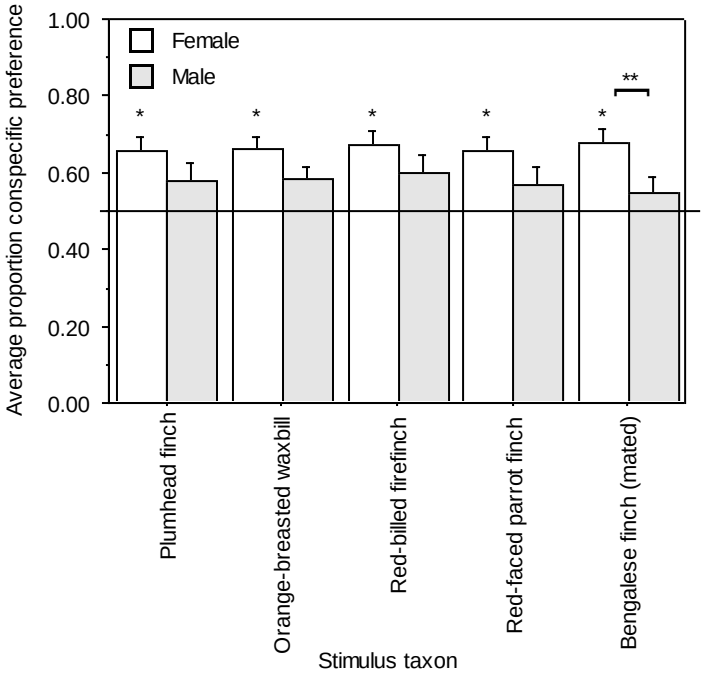


Figure 4.3: The average proportion of total 'discrimination time' the female and male zebra finch preferred the conspecific in the presence of the five heterospecific stimulus species (data shown for mated Bengalese finch only). Mean of the raw proportional values + SE depicted. Single asterisk indicates significantly different from random choice of 50% as shown by the solid line and double asterisk indicates significant difference in stimulus taxon responses between females and males.

I also employed a GLMM analysis on individual subject-averaged proportions of directed-song only and on combined total proportions of calling and courtship behaviours (including directed-song) directed towards the conspecific and heterospecific females, with male subject identity and heterospecific stimulus taxa added as random effects. Based on previous findings (ten Cate 1982; Clayton 1987a; Galoch & Bischof 2007) it was predicted that male subjects would direct more directed song and combined calling and courtship behaviours towards the conspecific. In contrast to patterns of spatial discrimination, I found support for these predictions (directed song: $F_{1,107} = 7.61$, $P = 0.0068$ [towards conspecifics least squares means estimate $\pm$ *SE* = 0.04 $\pm$ 0.0061; towards heterospecifics *LSM* estimate $\pm$ *SE* = 0.025 $\pm$ 0.0061]) (combined behaviours: $F_{1,122.1} = 21.34$, $P < 0.0001$) (Fig. 4.4).

Using a further GLMM I analysed the difference between conspecific and heterospecific female-directed song only and female-directed combined calling and courtship behaviours

(including directed-song) for each heterospecific stimulus taxon (5 heterospecific taxon levels) with male subject identity added as a random effect. A *post hoc* analysis using a Student's t-test was carried out on the least squares means estimates of the response variables. Similar to expectations of spatial discrimination I predicted that there would be variation in the directed-song only and female-directed combined calling and courtship behaviours across the range of heterospecific stimulus species used in my study in comparison to the domesticated Bengalese finch.

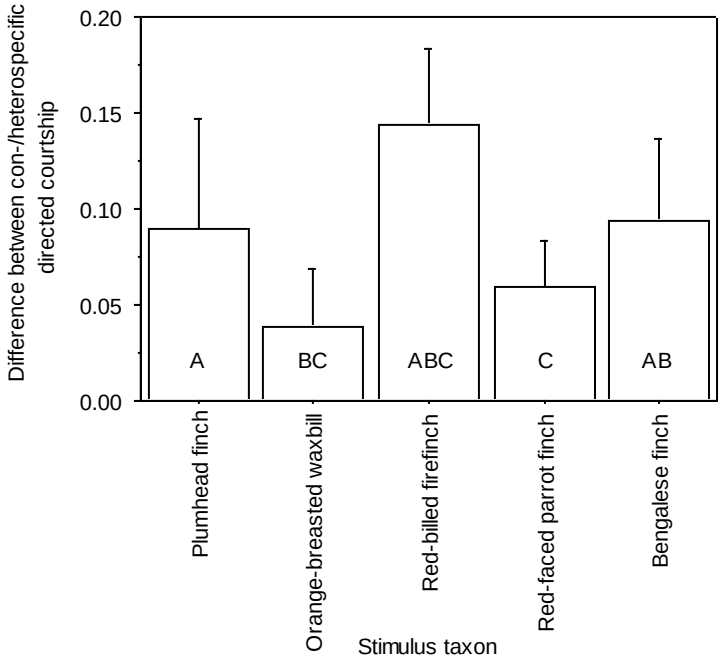


Figure 4.4: The average calculated difference between the proportions of conspecific-directed and heterospecific-directed combined calling and courtship behaviours in the presence of each heterospecific stimulus taxon. Mean of the raw proportional values + SE depicted. Species that are not connected by the same letter showed significant differences in the proportions of female-directed combined calling and courtship behaviours towards the conspecific and the heterospecific.

My results revealed that there was no variation in the calculated difference between directed-song towards conspecific and heterospecific females with respect to stimulus taxon ($F_{4,44}$ = 0.19, P = 0.95) but in support of the above predictions, there was significant positive variation in the calculated difference between the female conspecific stimulus-directed over the heterospecific-directed calling and courtship behaviours with respect to stimulus taxon ($F_{4,44}$ = 3.54, P = 0.014). However, *post hoc* tests showed that contrary to expectations, the Bengalese finch data were only significantly different from the red-faced parrot finch data

and the Australasian plumhead finch and red-faced parrot finch data were not both significantly different from the African red-billed firefinch and orange-breasted waxbill data (Fig. 4.4).

I also used GLMM on the log-transformed combined proportions of total time that the conspecific and heterospecific females spent calling and displaying courtship behaviours whilst located within the zone of closest proximity to the male, with male subject and heterospecific taxon added as random effects. It was predicted that the conspecific female would display more combined calling and courtship behaviours in the presence of a conspecific male compared to the heterospecific stimulus' behaviours (Sonnemann & Sjölander 1977) but contrary to this expectation I found no significant difference in the calling and courtship behaviours displayed by the conspecific and heterospecific females across all taxa ($F_{1,126} = 0.37$, $P = 0.55$) (Fig. 4.5).

To further assess the relationship between female stimuli's combined calling and courtship behaviours on the male subject's combined calling and courtship behaviours, linear regressions were carried out between the log-transformed proportions of total time for all taxa combined that the male spent calling and displaying courtship behaviours towards the conspecific females and the proportion of time the conspecific and the heterospecific females spent calling and displaying courtship behaviours across all taxa. Based on previous research (Collins 1994) it was predicted that there would be an interaction between the subject's and stimulus' behaviour although the causal direction of this relationship would remain uncertain because of the use of live stimuli. As expected, I detected significant interactions between the male subject's and female stimuli's calling and courtship behaviours. The male subjects displayed more calling and courtship behaviours towards the conspecific as the proportions of conspecific female calling and courtship behaviours increased ($R^2 = 0.07$, $P = 0.027$), and also as the proportion of heterospecific females' calling and courtship behaviours increased ($R^2 = 0.13$, $P = 0.0018$) (Fig. 4.5).

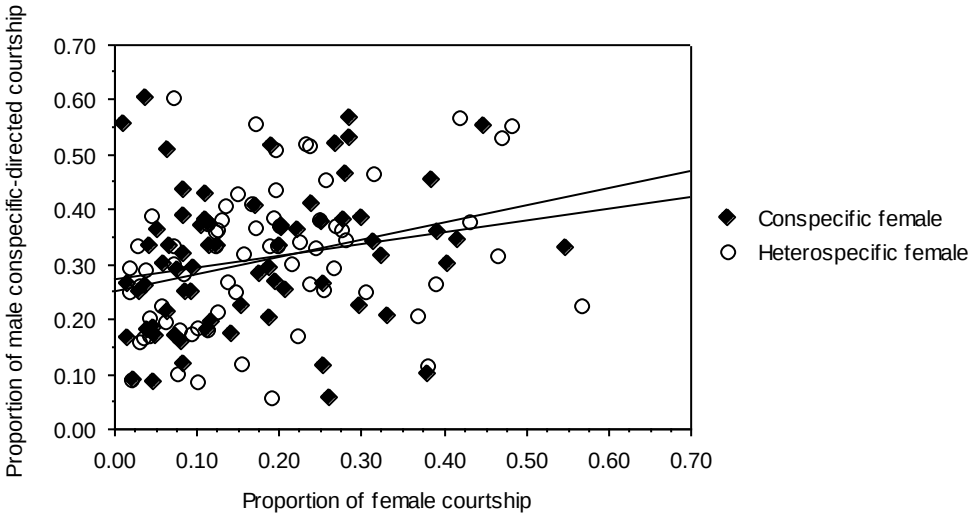


Figure 4.5: The relationship of the average proportion of total time that individual male subjects spent directing combined calling and courtship behaviours towards the conspecific in relation to combined calling and courtship behaviours displayed by the conspecific and by the heterospecific females across all heterospecific stimulus species combined. Individual data points indicate the trials (across both sets) for the 12 male subjects for all stimulus taxons.

To compare the effect of subject sex on the strength and consistency of conspecific discrimination GLMM was carried out on the 'discrimination time' spent in the spatial proximity of the conspecific for male data (this study) and female zebra finch data (from Chapter 3), with subject and heterospecific stimulus taxon added as random effects. Based on relevant theory (Andersson 1994; Wirtz 1999; Randler 2002) it was predicted that the male subjects would display a weaker preference for the conspecific than the same measure recorded previously for female subjects. This prediction was supported as I documented a statistically significant effect of sex; that is 'discrimination time' in the spatial proximity of the conspecific overall was greater for females than males ($F_{1,21.44} = 10.05$, $P = 0.0045$) (Fig. 4.3). Specifically, females spent more 'discrimination time' than males with the opposite sex conspecific.

To evaluate individual subjects' variation in the strength of conspecific preference, unpaired non-parametric Mann-Whitney signed-rank tests were also carried out between raw averages of proportional measures of conspecific spatial preference displayed by male (this study) and

female (Chapter 3) zebra finches for each of the given heterospecific stimulus taxons. It was predicted that there would be stronger discrimination displayed by the female, in comparison to the males, especially against the biologically more relevant heterospecific taxa. But results of the Mann-Whitney signed-rank tests showed the male and female subjects' preferences did not differ in response to each heterospecific taxon (plumhead finch Z = -1.73, P = 0.083; orange-breasted waxbill = -1.67, P = 0.094; red-billed firefinch Z = -1.42, P = 0.16; and red-faced parrot finch Z = -1.72, P = 0.085) except for the Bengalese finch (Z = -2.54, P = 0.011) (Fig. 4.3).

Finally, to explore the effect of sex on conspecific discrimination behaviour (i.e., 'sampling'), the average proportions of 'discrimination time' that the subjects spent in each zone were taken separately for both male and female subjects and the difference calculated between the average total time spent in zone 1 and zone 3 (irrespective of conspecific or heterospecific stimuli) for each heterospecific stimulus taxon. If the subjects were equally sampling both sides of the test cage then such a difference would be equal to zero. Unpaired non-parametric Mann-Whitney signed-rank tests were calculated between the average zone difference between male and female subjects for each heterospecific taxon. It was predicted that if the male subjects displayed a weaker conspecific preference than the female subjects then they might be less likely to sample both sides of the test cage. I confirmed a statistically significant sampling difference between the sexes for each heterospecific stimulus taxon with females sampling more the proximity of males than vice versa; (plumhead finch, orange-breasted waxbill, red-billed firefinch and red-faced parrot finch Z = -4.16, P = < 0.0001), with the exception of the Bengalese finch (Z = -0.98, P = 0.33) (Fig. 4.6).

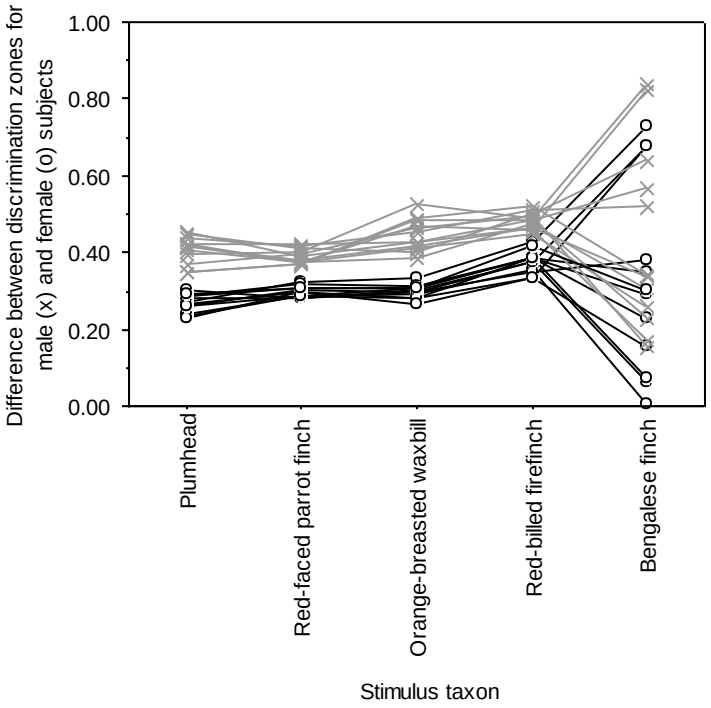


Figure 4.6: The average difference between total time spent in the discrimination zones (1 and 3) for male (x) and female (o) zebra finch subjects for each heterospecific species (only mated Bengalese finch data is presented). Individual data points indicate the trials (across both sets) for the 12 subjects for each stimulus taxon.

4.4 Discussion

These results on captive-bred male zebra finches in the context of species recognition, using a range of experimentally novel, biologically relevant heterospecific taxa, have revealed several contrasting findings. Some of the results using spatial and behavioural measures of species recognition are consistent with predictions of sexual selection theory, as well as with empirical data from previous social choice experiments on captive zebra finches. Other results on males' spatial proximity and courtship displays, however, are contrary to what was expected under phylogeographic theory and costly outcomes of hybridisation risk in a biparental species. Nonetheless, all may be important for the interpretation of past and future experimentation on social behaviour and its neurobiological basis in zebra finches as a model species for neuroethological research (Ziegler & Mahler 2004).

Specifically, using the simultaneous choice paradigm employed here, captive-bred male zebra finches did not spatially discriminate between conspecifics and heterospecifics, regardless as to what species was presented (Fig. 4.3). However, when measuring additional directed song and then combined calling and courtship behaviours there was a significant recognition and preference for the conspecific. Although the behaviours measured indicated consistent conspecific preference (Fig. 4.4), the zebra finch males' courtship behaviours were not exclusively directed towards the conspecific female stimuli. This is in contrast to previous studies that have found an almost exclusive song presentation and therefore sexual preference towards the conspecific (ten Cate 1982, 1985; Clayton 1987a).

Males of each species are predicted to be less choosy than females and might be less discriminating with their sexual displays (Andersson 1994), but it is also possible that different origins of stock and further domestication of captive zebra finches over the last 20 years, compared to many of the previous studies on captive zebra finches, could have altered the birds' discrimination behaviour. Accordingly, captive-bred female zebra finches in the same testing paradigms have recently been shown to behave differently from their wild counterparts (Rutstein et al. 2007). Additionally, different testing procedures between this and previous studies may have lead to the discrepancy in behavioural findings. I also acknowledge that with only two exemplars of each heterospecific taxon there may be constraints of pseudoreplication with the results obtained (Kroodsma et al. 2001). Although the males statistically showed no spatial or behavioural differences towards the two female

individuals from each heterospecific taxon, the acquisition of and testing with more than two representatives would increase the robustness of my results, a possible avenue for further experimentation.

Spatial patterns of social discrimination between live stimuli are notoriously difficult to interpret solely in the context of species recognition and mate choice (Göth & Hauber 2004), especially because zebra finches in the wild and in captivity also form mixed species flocks for foraging (Zann 1996). Nonetheless, my evidence on (the lack of) male spatial discrimination patterns is somewhat surprising, as this is a metric that has been used in previous male mate choice and social preference studies to detect consistent social preferences (Burley 1986; Burley & Coopersmith 1987; Jones et al. 2001 but see Witte & Sawka 2003; Witte & Caspers 2006) and is frequently applied especially in mate preference research on females (Sonnemann & Sjölander 1977; Wynn & Price 1993; Collins 1994; Brazas & Shimizu 2002; Forstmeier & Birkhead 2004). Additionally, this measure of spatial association indicated a consistent preference for the conspecific in my own previous study using female zebra finches (Chapter 3). However, in a recent sequential, rather than simultaneous, conspecific preference study, spatial association also failed to provide evidence for the species recognition preferences that the measured display behaviours revealed (Galoch & Bischof 2007). There is also the consideration that the large size of the stimuli cages that permitted greater flexibility in the spatial movement of stimulus females in comparison to previous studies (Sonnemann & Sjölander 1977; Wynn & Price 1993; Collins 1994; Brazas & Shimizu 2002; Forstmeier & Birkhead 2004) may have in turn influenced the males' motivation for spatial movement. This may have contributed to the lack of spatial preference for conspecifics but still permitted behavioural preferences in the measures that included acoustic components (calling and singing) which are used to maintain contact when the visual component is reduced (Zann 1996). Nevertheless, following predictions of sexual selection theory (Burley 1977) and consistent with previous experimental findings from zebra finches (Witte & Sawka 2003; Witte & Caspers 2006), my spatial measures of proximity were significantly lower for males than for female zebra finches using the same spatial metric (Chapter 3) (Fig 4.3). Thus, these results are consistent with experimental findings from zebra finches and other taxa that indicated the significantly more common role males played in the occurrence of hybridisation (Tynkkynen 2008) and the lesser extent of species (Dale & Slagsvold 1994; Luddem et al. 2004) and intraspecific discrimination (Witte

& Sawka 2003; Witte & Caspers 2006) shown by males in comparison to females of the same species.

The behavioural preference patterns for the conspecific female stimuli, however, were as expected, and also predicted by past work using measures of directed-song only that has been a preference indicator (ten Cate 1982, 1985; Clayton 1987a). In my study multiple behaviours were combined to give a more comparable indication of behavioural displays, discrimination, and the possible sexual motivation behind such preference. These behaviours included directed-song but were not limited to it as the preceding non-vocal displays that I measured are an important part of the courtship process (Morris 1954; Caryl 1976; Goodwin 1982). Unlike my measures of spatial association and directed-song alone, the behavioural displays indicated a significant difference in the strengths of conspecific preference depending on which heterospecific taxon was presented. I found some significant behavioural differences but not all of my phylogeographically relevant species elicited a stronger conspecific preference than the domesticated Bengalese finch.

In addition to the spatial and behavioural difference between males and females (see above, Fig. 4.3), the analysis of the extent of the spatial 'sampling' between the sexes produced interesting results in that males were significantly more biased to a cage side and did not sample the entire cage space as readily as the females did (Fig. 4.6). This was also noticeable in the 100 % side biases that some male subjects presented that did not occur within the female subjects. This lack of equal sampling could be due to a lack of motivation and/or lower strength of conspecific preference and lower activity levels in males (Ratcliffe & Boag 1987). Early work by Immelmann (1959) demonstrated significant differences in male sexual drive as did Brazas and Shimizu (2002) in their selection of only stimulus males that had high activity levels. It is also possible that the males' sampling was modulated by the risk of intersexual aggression in the presence of unfamiliar mated females (Dunn & Zann 1997), and they were therefore not as ready to move around the testing cage.

Interestingly, there was no significant difference in the sampling behaviour between male and female subjects in the presence of the Bengalese finch as individuals of both sexes showed the most extensive variability in their 'sampling' behaviours in the presence of this domesticated species compared to other heterospecific stimulus taxa used in my study (Fig. 4.6). Bengalese finches are the widely used heterospecific stimulus for experimentation with

captive zebra finches (Zann 1996) and the results of my studies indicate this species stimulates the most extensive test cage side-sampling bias for both zebra finch males and females (Fig. 4.6), reducing sex-specific differences and increasing individual variation in subject behaviour. This suggests that the use of such a domesticated heterospecific stimulus in comparison to other naturally occurring heterospecific stimulus taxa induces the least motivation for equal spatial sampling. This has implications for the applicability and use of Bengalese finches only, when looking at species discrimination in the laboratory.

The dynamic behavioural cues presented by the stimulus females may also have aided in conspecific recognition as these are an important part of the courtship process between the male and female zebra finches (Zann 1996). Although the behavioural measures employed did not find a significant difference between the stimulus females this is potentially due to an inaccurate measure of the heterospecific courtship. Early research suggests courtship behaviours do not significantly vary in species of Australian estrildines (Steiner 1955; Zann 1976). However, the pool of information on the highly diverse stimulus females that were used in this experiment needs to be substantiated before conclusions can be made. Nevertheless, the behavioural displays of the stimulus female conspecifics were positively correlated and those of the heterospecifics were negatively correlated with the behavioural displays of the male subjects. This suggests that the behaviours presented by the stimulus females were important for the process of preference decision-making.

Overall, my discrimination trials with captive-bred male zebra finches and a range of heterospecific stimulus taxa demonstrate that the type of heterospecific stimulus species used may affect various behavioural but not spatial metrics of the strengths of conspecific preference. Male zebra finches' behavioural responses covaried with the behaviours of female stimuli. Spatial proximity scores of male zebra finches showed significantly less discrimination against heterospecifics compared to female zebra finches' responses to opposite sex stimuli. These findings should be considered in future studies using zebra finches and other heterospecifics as a laboratory model system for the investigation of evolutionary and proximate factors involved in species recognition (Theunissen et al. 2004).

5

Behavioural correlates of female zebra finches' (Taeniopygia guttata) responses to playbacks of multimodal species recognition cues

5.1 Introduction

The ability to recognise conspecifics and to discriminate against heterospecifics is particularly important for sexually reproducing taxa which occur in phylogenetically closely related species assemblages (Price 2008). To avoid costly hybridisation these related sympatric species are expected to display greater discriminatory abilities than unrelated species in allopatry (Dobzhansky 1940; Coyne & Orr 1989; Kirkpatrick & Ravigné 2002), supported by findings in several lineages of animals, including *Drosophila spp.* (Coyne & Orr 1989, 1997; Noor 1995), midwife toads (*Alytes* spp.) (Márquez & Bosch 1997) and Darwin's Finches (*Geospiza spp.*) (Ratcliffe & Grant 1983) (but also see Ritchie et al. 1989; Collins & Luddem 2002). Therefore, when investigating the behaviours involved in the species recognition process, it is preferable to use phylogeographically relevant, sympatric species as research subjects/stimuli because such species are predicted to have evolved the sensory and behavioural traits required for accurate discrimination (Göth & Hauber 2004).

The recognition of conspecifics in most birds is suggested to be based on the combination of species-specific visual, acoustic, and olfactory cues (direct recognition) and spatial and temporal contexts (indirect recognition) (Grant & Grant 1997; Hauber et al. 2001). The behavioural mechanisms of avian species recognition and mate selection are widely studied (Hauber & Sherman 2001; Price 2008), particularly in females and in the popular model species of the sexually dimorphic, socially monogamous, zebra finch (*Taeniopygia guttata castanotis*) (reviewed in Zann 1996). To further understand the relative strength and salience of multimodal phenotypic cues that are being used for accurate conspecific recognition (Göth & Hauber 2004), I carried out laboratory experiments to determine female subjects' spatial and behavioural responses to conspecific and phylogeographically relevant heterospecific stimuli.

Based on acoustic cues or song playbacks alone, female zebra finches were previously shown to prefer males of their own vs. different subspecies (*T. g. guttata* of the Lesser Sunda Islands; Clayton & Pröve 1989; Clayton 1990b, c, d) and con- vs. heterospecifics, including the canary (*Serinus canaria*: (Lauay et al. 2004), or starling (*Sturnus vulgaris*: Braaten & Reynolds 1999). Surprisingly, to date there is little published evidence for con/heterospecific discrimination based on visual cues alone. Female zebra finches will approach painted male conspecific models of varying colour mutations (Immelmann 1959), but Brazas and Shimizu

(2002) reported that female zebra finches did not show behavioural discrimination between a live male conspecific and a live male heterospecific Bengalese finch (*Lonchura striata* vars. *domestica*) when only visual cues were transmitted to subjects. However, that study did not include a comparison of stimulus presentations including con- and heterospecific auditory cues, and looked at species recognition using a heterospecific domesticated finch stimulus species (the Bengalese finch) which is phylogeographically distant from the zebra finch amongst the estrildid finches (Sorenson et al. 2004; Chapters 3, 4).

A possible methodology for examining the salience of visual and acoustic features is through the use of video playback techniques. Video presentations allow both the consistency and manipulation of images that are potentially impossible to create with live stimuli (D'Eath 1998). Accordingly, this technique was used to study how female zebra finches' responses varied to video playbacks of individual conspecifics when auditory cues were swapped between stimulus birds (Galoch & Bischof 2006). In addition, the masking of auditory cues documented a significant preference for the visual display of an unfamiliar male conspecific over that of a familiar image of the females' mate (Swaddle et al. 2006).

To advance current knowledge, I applied video playbacks to assess the relative importance of the visual and acoustic cues involved in species recognition in captive-bred female zebra finches. In my work I selected a heterospecific sympatric, related stimulus finch species, the plumhead finch (*Neochmia modesta*) (Sorenson et al. 2004). Like the zebra finch, the plumhead finch is sexually dimorphic, endemic to Australia and is thus both ecologically and phylogenetically relevant to the native range of the zebra finch in its natural habitat (Zann 1996; Higgins et al. 2006). My methodology specifically involved playbacks of con- and heterospecific stimuli using acoustic only, visual only, and acoustic and visual cues combined, both in a species-typical match and mismatched between the acoustic and video playbacks.

Research to date also demonstrated several cases in which female zebra finches significantly individually vary in preferences for specific features in mate quality tests using conspecific stimuli (Burley & Coopersmith 1987; Riebel 2000; Forstmeier & Birkhead 2004). Such individual differences were also found to be consistent within across repeated trials in time (Riebel 2000; Forstmeier & Birkhead 2004). Independent of preferences, Forstmeier (2004) reported that female zebra finches varied significantly in their propensity to approach

stimulus males, the amount of overall time they spent with the presented stimuli (Forstmeier & Birkhead 2004), or the proportion of time they spent sampling different individual males during mate selection (Forstmeier et al. 2004). But there is little evidence of the stability of individual behavioural variation across different testing or functional/social contexts and how this might relate to eventual pair formation in this socially monogamous species. Research to date quantified how closely behavioural traits of males, including singing (ten Cate 1985; Tomaszycki & Adkins-Regan 2005) and social dominance (Ikebuchi & Okanoya 2006) correlate with pair bonding in a free-flight context. The direct physical attributes of female, including weight (Ikebuchi & Okanoya 2006) and perceived fecundity (Monaghan et al. 1996) are also predictors of mate bonding decisions. But there is limited evidence of indirect female behavioural attributes, including consistency of individual's choosiness and the role that such behavioural variation might play in eventual pair formation. To investigate such behavioural variation I also quantified individual female subjects' activity levels towards the playbacks of videos of con- and heterospecific males in my experiments. I measured the relative time spent clearly watching, and being engaged by the visual playbacks which I have termed 'attentiveness'. I then aimed to observe patterns of pair formation between unfamiliar males and subject females and quantified the relationship between individual behavioural variation and eventual pair bonding patterns.

Overall, in my experiments I asked the following five research questions:

1). *Does the female zebra finch recognise and prefer male conspecifics for different sets of potential species-recognition cue types presented?*
2). *Does the strength of preference vary between the different recognition cue types available to the female?*
3). *Does the female prefer the acoustic or visual cues more when these are mismatched between the stimulus species?*
4). *Do individual females vary in the strength of their attentiveness in relation to visual recognition cues available for species discrimination?*
5). *Does the variation seen in female behavioural attentiveness documented in playback tests of species recognition correlate with patterns of pair bonding in a free-flight aviary environment?*

5.2 Methods

5.2.1 Study subjects

The Australian subspecies of the zebra finch (*T. g. castanotis*) (length: ~ 10.5 cm, weight: 12 g) and the plumhead finch (length: ~ 13 cm, weight: 12.5 g) are both endemic to and in part sympatric in Australia (Zann 1996; Higgins et al. 2006) with the males of the two species differing in their songs and plumage colouration (Fig. 5.1).

As stimulus birds for recording the video playbacks I purchased six, unfamiliar plumhead finch males from local breeders in Auckland, New Zealand, and I obtained six unfamiliar wild type zebra finch males from the University of Auckland's outdoor breeding aviaries where all birds were raised in single-species aviaries by their genetic parents. Two unfamiliar wild-type female zebra finches were also obtained as training stimuli. All stimulus birds were colour-banded red on one leg for equal attractiveness to females (Burley et al. 1982) and were captive-sourced birds because of an importation embargo on wild bird species into New Zealand. For the free-flight aviary pair bonding trials (see below) I used 8 visually but not acoustically unfamiliar, unmated zebra finch males that had been raised in the University's aviaries (colour morphs: wild-type n = 4, fawn n = 4), all colour-banded red on one leg..

For test subjects I used 12 unmated (at the time of experimentation), adult female zebra finches of the wild type (n = 8), fawn (n = 3) and white (n = 1) colour morphs. Subjects were raised in the University's aviaries with social contact limited to conspecifics of wild-type, fawn and white colour morphs and were naïve to experimentation. Previous research showed no imprinting on parental wild-type and white morphs for subsequent colour morph conspecific mate preferences (Walter 1973 but see e.g., Burley 2006 for imprinting on artificial parental crests) and no difference in species discrimination responses, including zebra finch vs. plumhead finch by female (Chapter 3) or male zebra finches (Chapter 4). Only 11 of the subject females were used for the free-flight aviary behavioural experiment as one unknown-aged female died due to apparently natural causes in my aviary after the video playback trials. These females were identified by numbered black leg bands (Burley et al. 1982) on one leg (but could also be individually identified by sight by the observer [DLMC] during the pair bonding trials).

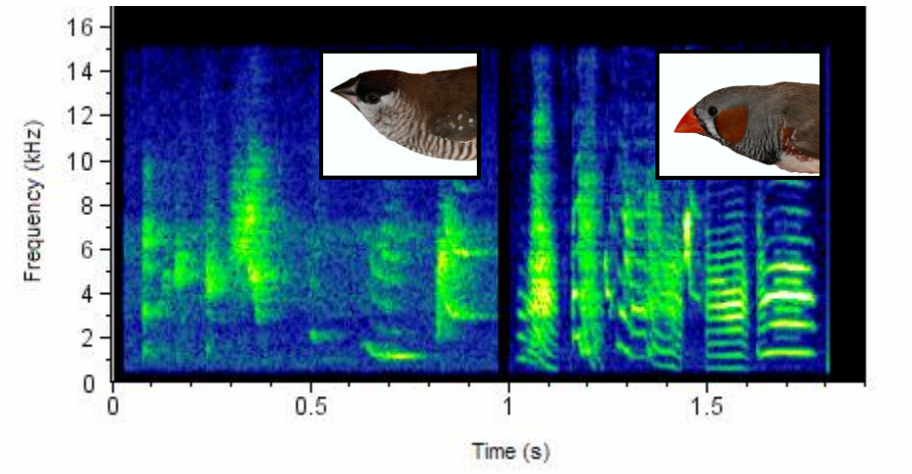


Figure 5.1: Profile images and frequency versus time spectrograms of single song motifs from the stimuli used in my experiments, plumhead finch males (left) and zebra finch males (right). [Photos: A. Campbell]

5.2.2 Animal housing and care

Female zebra finch subjects were housed on the University of Auckland, City Campus in a controlled room on a 16/8 hrs light/dark cycle, including a compact Arcadia^{TM} fluorescent bird lamp 2.4 %UVB and 12 %UVA with 35-65% daily humidity at 21 $\pm$ 3 ^{o}C (size of cages: 1.0m x 0.6 m x 1.8 m). The female test subjects in a single sex group were visually but not acoustically isolated from other breeding zebra finches. All stimulus males for video playbacks were held in species separate, single sex groups in remote outdoor aviaries and were brought onto campus for filming for a single day each. The unmated males for the free-flight pair bonding were housed together in a single-sex cage in visual isolation from the stimulus females.

All birds were fed *ad libitum* a commercial finch dry-seed mix **(**Animates^{TM}**)** with fresh water replenished daily and spray millet, fresh cucumber, corn, rice and boiled hen's egg given weekly. Calcium, iodine and grit supplements were also provided. All birds were monitored daily and housing and experimental protocols were approved by the University of Auckland Animal Ethics Committee. All birds were placed in private aviaries on completion of experimentation.

5.2.3 Preparation of playbacks

To generate the video stimuli, the zebra finch (n = 6) and plumhead finch males (n = 6) were individually placed in a small cage (length 0.48 m, width 0.30 m and height 0.30 m) to limit axis depth variation (Oliveira et al. 2000), with a clear Perspex front and a single perch running the length of the cage. An unfamiliar female zebra finch was placed in front of them (but behind the camera) for courtship stimulation.

The filming took place in sound proof, fluorescently-lit animal housing cabinets closed to human interference during recording. The males were filmed with a Sony DCR-HC40E Pal Digital Camcorder that was placed so that the males performing their courtship towards the female (not present in any footage) would be captured as performing towards the camera. The males were filmed from a distance so as to be life-sized when played back on the LCD screens (Oliveira et al. 2000) and only footage of courtship activity at the front of the cage, nearest to the camera, was used. This courtship activity, including directed song, beak-wiping, hopping, pivoting and tail twisting (Caryl 1976; Zann 1996) was edited using Windows Movie Maker and saved as highest quality DV-AVI files to make 10-min videos for playback. An equal proportion of directed song was included in each stimulus video (24 singing bouts at varying intervals during the 10 min playbacks ranging from 2-5 motifs). A Marantz PMD 671 solid state recorder with a Sennheiser ME66 shotgun microphone was used to simultaneously record the males' song at a sampling rate of 44.1 kHz and 16-bit resolution digitised directly as a wave file.

The acoustic recordings for each male were bandpass-filtered at 500-15000 Hz using Raven Sound Analysis Software v.1.2.1 ™ Cornell Laboratory of Ornithology (Charif et al. 2004) and a soundtrack to accompany the video footage was compiled that included only the male song matched to the visual singing behaviour and silence during all other courtship behaviours. All male and female calls were removed as only the female response to male song was being assessed. An additional set of video playbacks was made in which the acoustic soundtracks had been swapped so that when the male zebra finch was seen singing, a plumhead finch song was broadcast, and vice versa.

Acoustic-only files for both species were also created in Raven v. 1.2.1 that included male song at a higher rate (48 singing bouts at varying intervals during the 10 min playbacks ranging from 2-5 motifs per bout) than the video files so as to provide higher stimulation in the absence of continuous video images. In addition to the filming of the male stimuli, two unfamiliar female zebra finches were filmed to be used as training videos for the test subjects.

5.2.4 Set-up of discrimination trials

The testing apparatus and spatial discrimination zones are shown in Fig. 5.2. Two Dell latitude D620 laptops with LCD screens of 1440 by 900 spatial resolution were placed in front of P (perch) 1 and P5 at a distance so that the screens were 0.45 m from the cage and were visible from P2 and P4 but not from P3 (Fig. 5.2). Acoustic playback output was set at 60-68dB, as measured at the centre of P1 and P5 using a digital sound level meter (Radioshack 33-2055). Food and water *ad libitum* were placed in the neutral zone of the cage, opposite P3. A fabric screen with mesh holes, positioned 1.0 m from the test cage permitted the observer to watch and record all trials unseen (Fig. 5.2).

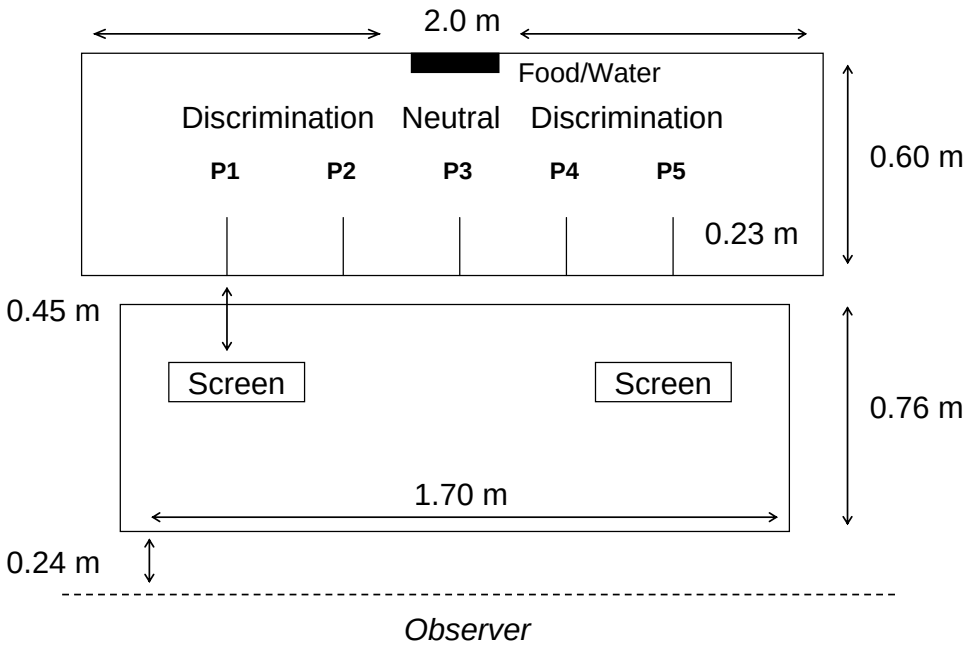


Figure 5.2: The testing apparatus as viewed from above consisted of a single female subject cage (length 2.0 m, width 0.6 m and height 0.8 m) at 0.87 m above the ground. The cage was divided with 5 perches (length 0.23 m, placed at height 1.12 m above ground) in which 2 were placed 0.53 m from the cage ends and the other 3 evenly distributed across the space in between. These perches were labeled (P) 1-5 with P1, P2 and P4, P5 (and the immediate surrounding ground area; P1 and P5: 0.65 m, P2 and P4: 0.235 m) classed as discrimination zones and P3 as a neutral zone (including immediate ground area; 0.235 m). A table clothed in white fabric (1.70 x 0.76 x 0.93 m) was placed 0.05 m from the test cage. Food and water is depicted in the neutral zone and the dashed line represents the screen behind which the observer was positioned.

5.2.5 Experimental procedure

Experimental playback trials were run daily from February-April 2007 starting at 12 midday (NZST) and adhered to the same time schedule across trials. The lighting in the experimental room was fluorescent with an ambient temperature of 20 $\pm$ 2 ^{o}C. Each female test subject was initially placed in the test cage for one day prior to the start of all trials to allow 24 hr to become accustomed to the new surroundings. During this time they were exposed to 3 hr of silent simultaneous playback of two female zebra finches to become familiar with the video images.

The female subjects were each placed in the test cage immediately before their own trials and given 30 min to again familiarise themselves with the surroundings. This procedure enabled several female subjects to be tested during a single day as a maximum of 4 trials were run in sequence with each subject to minimise a possible reduction of social/sexual motivation for spatial choice behaviours.

All data were collected by the same observer (DLMC) using methods that showed consistent interobserver levels of species discrimination patterns by female zebra finches (Chapter 3). Simultaneous visual playbacks and/or alternating sound tracks (so as not to overlap the acoustic cues) were presented on the two screens. Each female was presented with a sequence of four 10-min playbacks randomly representing two of the stimulus types (see below), with 15 min breaks in between. Side presentation of male stimuli was counterbalanced between each pair of trials for the particular stimulus type. The location (P1, P2, P3, P4 or P5) and behavioural displays (Zann 1996) of the female were recorded using a scan-sampling design every 10 s (one record of both current location and behavioural stage). For the behavioural observations the proportion of time the females spent clearly watching the video screens, ‘attentiveness’ was noted as a behavioural measure of individual consistency.

The female subjects were presented with one of five different stimulus playback types:

1. *Acoustic Only*
2. *Visual Only*
3. *Acoustic & Visual Combined for the Two Finch Species*
4. *Acoustic & Visual Swapped between the Two Finch Species*
5. *Zebra Finch Acoustic & Visual vs. Zebra Finch Acoustic & Plumhead Finch Visual*

To reduce pseudoreplication owing to behaviours of individual stimulus males, all female subjects were observed in response to playbacks recorded from two different conspecific and heterospecific individuals for each of the stimulus playback types (following Hauber et al. 2001). In contrast, due to a limited number of male plumhead finches available to me, the same male (matched with the same male zebra finch for equality) was used to present the *Acoustic & Visual Combined* then the *Acoustic & Visual Swapped* stimulus types. Overall, each female subject was observed over four 10-min trials for each of the five stimulus playback types and, therefore, each subject was observed over 20 trials (200 min) in total.

5.2.6 Set-up of free-flight aviary trials

Observations of behavioural interactions in free-flight aviary trials were run from July-November 2007 in a single cage (2.0 m x 0.6 m x 1.67 m). All behavioural trials were documented by a single observer (DLMC) through a glass-windowed door without being seen by the subjects (i.e., no discernible change in behaviour upon arrival of the observer: DLMC pers. obs.).

5.2.7 Experimental procedure

Prior to any behavioural observations being made, a visually unfamiliar, unmated male zebra finch was added to the female cage (containing all 11 females) and left for 5 days (latency criterion to document pair bonded behaviours, Zann 1996). Behavioural observations were made for 1 hr each day for the next 5 days and, for consistency, all data were collected during the evenings; at approximately 7-8 pm local time as this was the time that male-female interactions were most prominent (DLMC pers. obs.). A 30 s scan sampling design was employed to record the behaviour of the male. If the male engaged in pair bonding

behaviours with any of the 11 females, this female's corresponding behaviour and identity were also noted. It was predicted that if females' attentiveness behaviours were related to stable behavioural traits then this variation would correlate with the females' engagement in pair bonding behaviours across all of the presented males. The alternative would be that random patterns of pair bonding were observed for each female.

The behavioural interactions between female and male stimuli included clumping (sitting very close to each other), allopreening, and directed singing of which allopreening and clumping are the strongest indicators of a pair bond formed and were used as criterion here (Zann 1996). Aggressive interactions (n = 7) and copulation behaviours (n = 1) were too infrequently witnessed to include in any analyses. After five days of observations the male was removed and the females were left for 3 days, simulating the maximum time a female would be separated from her mate in the wild (Miller 1979a), before the next stimulus male was introduced. The females were also weighed to the nearest 0.01g (Mettler AE 166^{TM} electronic scales) on two occasions to obtain their mass as a potential predictor of female quality (Ikebuchi & Okanoya 2006).

5.2.8 Data analyses

For the discrimination trials, the spatial data were analysed to calculate a total 'discrimination time' for zebra finch stimulus types by dividing the time spent in the proximity of the zebra finch cues by the total amount of the time spent in the proximity of either stimulus combined (total time spent in P1 and P2 collectively and P4 and P5 collectively in each trial) per trial (Hauber et al. 2000, 2001). The exception was that I calculated discrimination time for the trials of '*Acoustic & Visual Swapped*' as the relative time spent with the zebra finch acoustic (and plumhead visual) stimulus. I then calculated average discrimination times across the four trials for each stimulus playback type so that each female subject contributed a single datapoint per stimulus playback type to the analyses.

As some of the subjects did not sample both video screens during some of the 10-min trials, any first trial of a stimulus playback type in which 100% of the total 'discrimination time' was spent only on one cage side was eliminated from calculating averages but all subsequent trials were retained for analyses when the female would have been exposed to both stimuli per trial type. One-sample t-tests revealed significantly less time than random expectation

0.079 ($\log_{10}(0.2+1)$) spent in the neutral zone for all stimulus playback types (t_{11} all $P \leq$ 0.0041) except for the *Visual Only* stimulus type (t_{11} = -1.99, P = 0.072), although this was due to a single subject that did not approach either of the playback screens (with this female excluded: t_{10} = -5.72, P = 0.0002). These behavioural patterns indicated the subjects were engaged by the stimulus presentations through spatial proximity. There were no significant differences among the proportions of time spent in the neutral zone for individual subjects across stimulus playback types as revealed by Generalised Linear Mixed Model analysis (GLMM) with individual identity added as random effect ($F_{4,44}$ = 0.43, P = 0.78). Total proportion of 'attentiveness' as the total proportion of 'discrimination time' spent oriented towards the video playbacks (either conspecific or heterospecific, combined) when in the discrimination zones, was also calculated for each subject for the three stimulus playback types that presented both visual and acoustic cues.

For the *Visual Only* stimulus type presented, the females did not readily approach or otherwise sample both screens as in all other stimulus types. Specifically, 50% of the female subjects were seen to spend 100% of their total 'discrimination time' on one cage side throughout all four *Visual Only* trials, while one subject did not approach the screens at all (i.e., remained in the neutral zone). Despite the initial training period, it was unclear whether the subjects were aware of both screens broadcasting images in the absence of acoustic cues. This stimulus playback type is included in the discrimination analyses but must be interpreted with caution. Additionally, due to the inconsistency in subject response towards the silent screens, the attentiveness behaviours of subjects during this stimulus playback type were not included in any analyses.

For the free-flight aviary data, the total count of all clumping, allopreening and directed singing interactions that each female engaged in, in relation to each conspecific male were summed. I then calculated the total proportion of these summed interactions that occurred with a particular female to determine as to which females most often formed a pair bond with the male.

All proportional data were log transformed ($\log_{10}(X + 1)$) and analysed by parametric tests to assess significance ($\alpha < 0.05$) using Statview 5.0.1 and JMP 7 (Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA). All tests were two-tailed. For illustrations I

present the raw values of female conspecific preference, average attentiveness, and pair bonding behaviours.

5.3 Results

For the discrimination trials, one-sample t-tests were used to assess conspecific preference for each of the five stimulus playback types with 0.176 ($\log_{10}(0.5+1)$) set as the random expectation. As predicted, female zebra finches showed consistent spatial association with the conspecific male stimulus above the random expectation of 50% of 'discrimination time' for both the *Acoustic Only* (t_{11} = 5.92, P < 0.0001) and *Acoustic & Visual Combined* (t_{11} = 6.03, P < 0.0001) stimulus playback types (Fig. 5.3). There was no significant spatial preference for the conspecific in the remaining experiments, including *Visual Only* (t_{10} = -0.52, P = 0.62), *Acoustic & Visual Swapped* (t_{11} = 0.47, P = 0.65), and *Zebra Finch Acoustic & Visual vs. Zebra Finch Acoustic & Plumhead Visual* (t_{11} = 0.51, P = 0.62) (Fig. 5.3).

These results were confirmed by GLMM analysis on the log-transformed proportions of discrimination time spent in spatial proximity of the conspecific (or conspecific audio in the *Acoustic & Visual Swapped* stimulus type) for each stimulus playback type, with female subject identity added as random effect. There was a difference in the extent of conspecific spatial association based on the stimulus playback type ($F_{4,43.75}$ = 4.48, P = 0.004) (Fig. 5.3). Specifically, a Student's t-test on the least squares means estimates showed no significant *post hoc* difference in the proportions of time spent in proximity of the conspecific between stimulus playback types *Acoustic Only* and *Acoustic & Visual Combined* or between *Visual Only*, *Acoustic & Visual Swapped* and *Zebra Finch Acoustic & Visual vs. Zebra Finch Acoustic & Plumhead Visual*. However, as expected, there was a significant difference in conspecific spatial association between stimulus playback types *Acoustic Only, Acoustic & Visual Combined* and the remaining three stimulus playback types (Fig. 5.3) (Table 5.1).

The GLMM analysis also showed significant differences in the attentiveness by female zebra finches across the three stimulus playback types ($F_{2,22}$ = 20.80, P < 0.0001), with the most time spent observing the videos for the stimulus playback type *Zebra Finch Acoustic & Visual* vs. *Zebra Finch Acoustic & Plumhead Finch Visual* (Fig. 5.4) (Table 5.2). Overall, a

one-way ANOVA showed significant interindividual differences in attentiveness during the discrimination trials ($F_{11} = 4.30$, $P = 0.0014$) (Fig. 5.5a).

Similarly, one-way ANOVA also showed a significant difference in the average proportions of total pair bonding behaviours that each female was recorded to be engaged in with the live conspecific males in the free-flight aviary ($F_{10} = 2.34$, $P = 0.018$). Linear regression indicated that the proportional measures of pair bonding behaviours in the free-flight context were significantly related to the attentiveness behaviours of the respective individual females in the discrimination trials ($R^2 = 0.47$, $P = 0.021$) (Fig. 5.5a). Specifically, females that spent greater proportions of time engaged in video-observing behaviour were more likely to engage in pair bonding behaviours with the males, when controlled for the weak positive relationship between individual females' involvement in pair bond behaviours and the respective weight of the females in my sample ($R^2 = 0.30$, $P = 0.08$) (Fig. 5.5b).

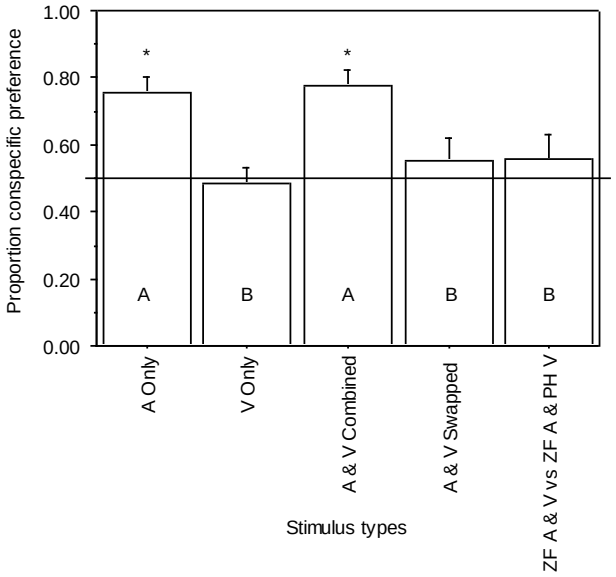


Figure 5.3: The average proportion of total 'discrimination time' the female zebra finch preferred the conspecific in the presence of the five stimulus types [A Only (*Acoustic Only*), V Only (*Visual Only*), A & V Combined (*Acoustic & Visual Combined*), A & V Swapped (*Acoustic & Visual Swapped*), ZF A & V vs. PH A & ZF V (*Zebra Finch Acoustic & Visual vs. Zebra Finch Acoustic & Plumhead Finch Visual*)]. Mean of the proportional values + SE depicted, the solid line indicates random choice of 50%, *asterisk* indicates significantly different from random expectations and stimulus types without the same letter showed significant *post hoc* differences.

Table 5.1: Summary output of the GLMM analysis on the female zebra finch proportion conspecific spatial association for the five stimulus types, showing least squares means estimates (*LSM*), standard error (*SE*) and degrees of freedom (*df*).

Stimulus type	***LSM* estimates**	***SE***	***df***
Acoustic Only	0.2431	0.016	11
Visual Only	0.1682	0.017	10
Acoustic & Visual Combined	0.2473	0.016	11
Acoustic & Visual Swapped	0.1855	0.016	11
Zebra Finch Acoustic & Visual vs. Zebra Finch Acoustic & Plumhead Finch Visual	0.1872	0.016	11

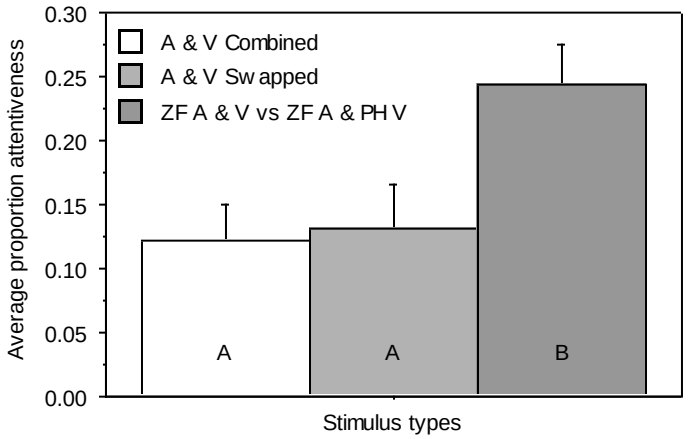


Figure 5.4: The average proportion of total 'discrimination time' all subjects spent observing the video presentations (attentiveness) for stimulus types that included both visual and acoustic components. Mean of the proportional values + SE depicted where stimulus types without the same letter showed significant *post hoc* differences.

Table 5.2: Summary output of the GLMM analysis on the female zebra finch proportion attentiveness for the three stimulus types, showing least squares means estimates (*LSM*), standard error (*SE*) and degrees of freedom (*df*)

Stimulus type	***LSM* estimates**	***SE***	***df***
Acoustic & Visual Combined	0.048	0.012	11
Acoustic & Visual Swapped	0.052	0.012	11
Zebra Finch Acoustic & Visual vs. Zebra Finch Acoustic & Plumhead Finch Visual	0.093	0.012	11

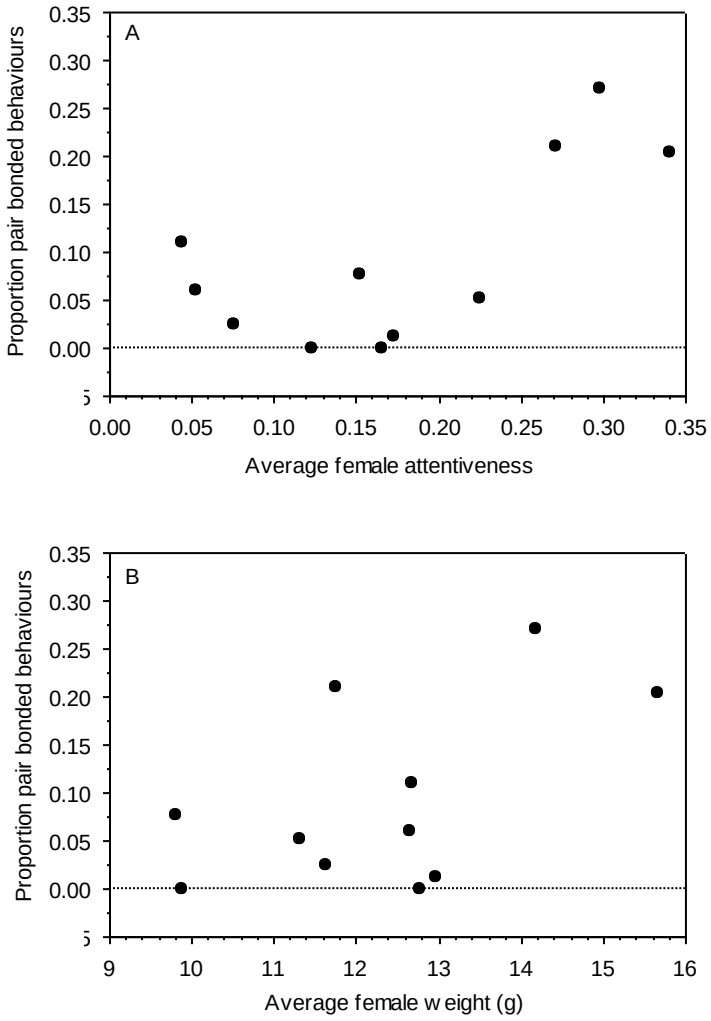


Figure 5.5a, b: The average proportion of total pair bonded behaviours that each stimulus female was engaged in with the stimulus males in relation to the average observed female attentiveness in the video playback trials (a) and average females' weight in grams (b). Mean of the raw proportional values depicted.

5.4 Discussion

The results of these experiments suggested consistent differences in the relative salience of the visual vs. acoustic cues in the recognition system of conspecifics by female zebra finches through the use of video and/or acoustic playbacks. Specifically, my findings (1) confirm and demonstrate, respectively, that species discrimination is paralleled by patterns of spatial proximity of captive-bred female zebra finches towards playbacks of (1a) acoustic and (1b) acoustic and visual species-specific cues of male conspecifics which are consistently greater compared to proximity towards cues of male heterospecifics of a phylogeographically relevant taxon. Furthermore, the results (2) suggest that acoustic cues are more salient signals of species identity than are visual cues, but also (3) imply that visual cues are incorporated into discrimination decisions based on multimodal sensory cues in the species recognition process.

Additionally, I documented a positive correlation between variation of the strength of individual females' behavioural attentiveness during responses in the species discrimination trial and the proportion of pair bonding behaviours with conspecific males in a free-flight aviary paradigm. This result implies both an individual consistency of female behavioural traits across testing contexts and confirms the relevance of my behavioural measures in the discrimination trials for the mate selection processes in zebra finches.

The low behavioural responses and inconclusive results of my *Visual Only* presentation are similar to previous research that found no discrimination by female zebra finches between con- and heterospecifics when only visual features were available through one-way glass (Brazas & Shimizu 2002). However, in my study there was the additional contributing factor that in the absence of auditory cues several females did not even sample both stimulus screens. It is possible that visual cues presented alone, in comparison to auditory cues alone, are an unusual social and ecological context that the zebra finch is unlikely to encounter in the natural habitat, therefore, resulting in the lack of subject response. Surprisingly, however, in my trials females also showed no significant conspecific preference in the stimulus playback type where they were exposed to consistent conspecific songs but two visual species. This is in contrast to the work of Brazas and Shimizu (2002) on conspecific discrimination with live male stimuli including Bengalese finches, where a mismatch of conspecific song with the conspecific visual cues did not eliminate the female subjects'

propensity to display preference for the conspecific. Female subjects in my trials may also have not sufficiently experienced that there was another screen to view. This would be consistent with differences in subjects' training and learning ability as has been documented in previous zebra finch experimental trials involving artificial (i.e., song playbacks, as opposed to live individuals) stimuli (Spencer et al. 2005), although in my trials these differences were eliminated when the acoustic stimulus was added to the video playback.

More critically, however, although video playbacks were successfully used in previous mate and species preference research using zebra finches (Swaddle et al. 2006; Galoch & Bischof 2006, 2007), I acknowledge that this playback medium likely led to a distortion of the species-specific UV signals presented, as perceivable by the zebra finch visual system (Cuthill et al. 2000) and, thus, in the extent to which these cues are used in zebra finch species recognition (Bennett et al. 1996; Hunt et al. 1997) relative to acoustic cues. The conspecific preference shown in response to the *Acoustic & Visual Combined* stimulus playback type was similar to female zebra finch preferences for live conspecific over plumhead finch males in trials previously carried out in a room that included limited UV spectra (Chapter 3). Additionally, the elimination of female zebra finches' species discrimination in a spatial paradigm in response to the experimental mismatching of visual and acoustic conspecific and foreign cues was also recently demonstrated by myself, using plumage manipulation (Hauber et al. 2000) and cross-fostering of live male conspecific stimuli (Chapter 6). But it remains possible that the video presentations did not present critical visual cues either in the UV spectrum or by the nature of the two-dimensional playback screens in the experiments analysed here. Further research with live stimuli or moving three-dimensional models (Patricelli et al. 2002) would be needed to confirm the relative salience of these different sensory signals for species recognition by the zebra finch.

In spite of the limitations of video playbacks and the lack of clear patterns of conspecific spatial association recorded in the *Visual Only* playback trials, decreased female conspecific preference was also observed when the visual and acoustic cues were swapped, relative to the consistent conspecific preference in the *Acoustic Only* and *Acoustic & Visual Combined* stimulus playback types. These patterns indicate that visual cues of species identity presented on the video screens do modulate species discrimination decisions in zebra finch females, similar to previous research on female choice behaviour between different conspecific males (Galoch & Bischof 2006). Thus, the swapping of the different sensory cues disassociated

relevant recognition cues for the females leading to no clear preference for either conspecific acoustic or conspecific visual cues.

Taken together, these patterns confirm that similar to recent zebra finch mate selection research (Holveck & Riebel 2007), acoustic cues alone are strong enough to elicit a preference response and that the visual stimulus was a redundant feature (Partan & Marler 2005). In support of this scenario, acoustic cues are used predominantly in the zebra finches' natural habitat to maintain contact with social partners in large foraging flocks and within mate bonded pairs at breeding colonies when a visual connection has been lost (Zann 1996). Visual cues in turn may have evolved primarily for intraspecific communication involving mate choice and reproductive investment (Gill et al. 1999). I suggest here that visual features are potentially less important than acoustic signals during the species recognition process but that when a mismatch occurs between acoustic and visual cues, there is a role for visual features to modulate species preferences by female zebra finches. Future work with male zebra finches involving the mismatch of species-specific female visual and acoustic cues is also required to address potential sex-differences and the role of social context on the relative salience of these sensory signals (Vignal et al. 2004).

My findings also demonstrate that there was a significant difference in the behaviour that individual females displayed toward the playbacks as measured by the proportion of time they spent clearly engaged by the male images and songs while in the discrimination zones. This attentiveness behaviour was in turn positively related to patterns of pair bonding with unfamiliar males, implying a functional consistency in a variable female behavioural trait across different contexts. This consistent behavioural variation is similar to recent findings from male zebra finches indicating correlations between song complexity and response to a problem-solving task, reliably indicating male quality (Boogert et al. 2008). It is possible that the attentiveness behaviours of the females were an indication of female choosiness, representative of the cognitive, temporal, and energetic investments that a female expends in mate selection decisions (Jennions & Petrie 1997), and were therefore also an indication of the quality of the female (Forstmeier et al. 2004).

There are many variables that may be involved in pair bonding decisions, and my behavioural observation procedure did not detail the possible roles of dominance or initial courtship behaviours in eventual pair bond formation. I cannot conclude for certain if males

were more attracted to the more attentive females or if attentive females were more aggressive in their pursuit of the males. Furthermore, although not significant, there was a positive trend in heavier females pair bonding more often suggesting additional factors were relevant. However, my experiments demonstrate that female behavioural responses in a species recognition testing context were highly correlated with their likeliness to establish pair bonds with conspecific males in a free-flight aviary paradigm. This correlation is suggestive of the role of individual consistency in a variable female behavioural trait in mate selection decisions. Measuring the role and benefits of repeatability of females' varying behavioural responses in other testing contexts and pair bonding behaviours of these females with unfamiliar males in a structured choice chamber paradigm is therefore also warranted in future investigations.

6

The disassociation of visual and acoustic conspecific cues decreases the strength of discrimination by female zebra finches (Taeniopygia guttata)

6.1 Introduction

Species recognition in birds and other sexually reproducing taxa is based on the accurate perception of visual and acoustic conspecific cues (Hauber et al. 2001). The zebra finch (*Taeniopygia guttata*) is a sexually dimorphic, socially monogamous, estrildid songbird (Zann 1996), in which the female uses males' visual features, including the colour of the beak (Burley & Coopersmith 1987), chest bands (Swaddle & Cuthill 1994), or cheek patches (Naguib & Nemitz 2007), and acoustic features of male song, including production rate (Houtman 1992), quality (i.e., tutored vs. untutored song, (Lauay et al. 2004), and species identity (Clayton & Pröve 1989; Clayton 1990; Lauay et al. 2004) for conspecific discrimination.

Female zebra finches can recognise conspecifics as suitable mates based solely on acoustic cues (Miller 1979a; Vignal et al. 2008) and recent experimental evidence suggests that acoustic features are most salient during preference decisions for certain classes of conspecifics (e.g., familiar individuals, social mates) (Galoch & Bischof 2006). Nevertheless, evidence from video playbacks also confirms that species recognition in zebra finches is multimodal and that the experimental mismatch of visual and acoustic cues both in the context of mate or species recognition can reduce female discrimination in a possible effect termed 'signal confusion' (Chapter 5; Galoch & Bischof 2006). But video playbacks do not show full spectrum colour of male visual displays (Cuthill et al. 2000) and have also been found to shift female preferences relative to the familiarity status of the presented male images, therefore fundamentally changing female behaviour (Swaddle et al. 2006). Here I set out to further extend the knowledge on the multimodality of conspecific recognition and the relative roles of visual and acoustic cues by female zebra finches. I used live male conspecific stimuli of two colour morphs, wild-type (conspecific) and white with a black-painted beak (foreign), producing one of two vocalisation types, normal-reared conspecific songs and calls (conspecific) or cross-fostered song and calls of males raised by Bengalese finches, *Lonchura striata* vars. *domestica*) (foreign). I then used this experimental mismatching of predicted 'preferred' visual and/or acoustic cues (conspecific) to quantify the salience of these cue types on conspecific social choices in a spatial discrimination paradigm.

6.2 Methods

6.2.1 Study subjects and stimuli

As study subjects I used 15 adult female zebra finches (wild-type colour morph n = 8; fawn n = 7) captive-raised by their genetic parents who were naïve to experimental testing with live stimuli. Subjects were housed in single sex cages visually and acoustically isolated from stimulus males. Details of housing are given in Chapter 3. As stimuli I used unfamiliar zebra finch males which were (i) wild-type colour morph, normally-reared males (n = 6) that produced species-typical social songs and calls, (ii) marked white colour morph, normally-reared males (n = 6) that had minimal plumage ornamentation (i.e., dulled cheek patches and chest band) and produced species-typical social songs and calls and whose beaks were painted with black nail varnish (Diamond Gloss # 87), to further reduce conspecific-typical visual cues, and (iii) wild-type colour morph (n = 6) cross-fostered males that produced Bengalese finch-type songs and calls (Zann 1985; Eales 1987) (Fig. 6.1). As controls for visual treatments, all wild-type normal-reared and cross-fostered males were nail varnish painted under the wing (Hauber et al. 2000) in a non-visible location to eliminate confounds due to exposure to dye and/or olfactory cues (Steiger et al. 2008). All birds were marked with a numbered pale pink band on one leg.

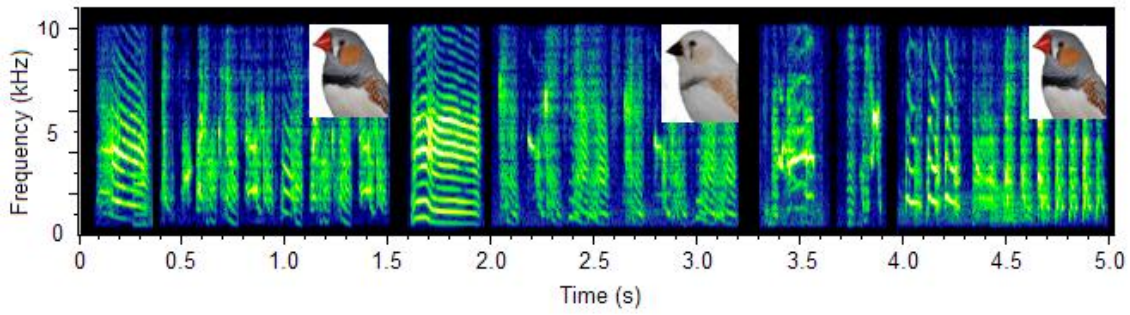


Figure 6.1: A spectrogram of representative male calls and songs and photo images of stimulus types showing from left to right, Wild-type normal-reared, White normal-reared, Wild-type cross-fostered.

6.2.2 Experimental set-up and procedure

Following previous work of mine that showed consistent patterns of species recognition in zebra finches using live stimuli (Chapters 3, 4), I used repeated trials of 10-min duration with several sets of stimulus males on my sample size of 15 individual females. Trials were run from 12 midday onwards (NZST) during October-December 2007, in a room lit by natural light with added fluorescent bulbs for increased brightness. The testing apparatus consisted of a single cage (length 2.0 m, width 0.6 m and height 0.8 m) placed 0.87 m from the ground and divided with 5 perches (length 0.23 m, placed at height 1.12 m) distributed evenly across the central 1.7 m of the cage space (Fig. 6.2). These perches were labelled (P) 1-5 with P1/P2 and P4/P5, together with their respective immediate surrounding ground area, classed as discrimination zones and P3 as neutral. A table clothed in white fabric (1.70 x 0.76 x 0.93 m) was placed 0.05 m from the test cage (Fig. 6.2). Two small cages (0.48 x 0.30 x 0.38) (evenly divided with two perches across the width) were placed in front (0.15 m) of the end perches and white plastic divides visually isolated the stimulus males from each other (Fig. 6.2). Subject females could not see both males simultaneously from any of the five perches and all birds were supplied with food and water for the duration of the trials. A fabric screen was used to visually isolate subject and stimuli between trials. All behavioural observations were made in real time by the same observer (DLMC) who remained unseen and all experiments were approved by the University of Auckland Animal Ethics Committee. This data collection approach did not require tests of interobserver repeatability and did not allow tests of intraobserver repeatability. However, in a previous study using the same methodology I found statistically indistinguishable patterns of spatial discrimination metrics between DLMC and another observer (Chapter 3) and spatial choice data collected by DLMC for this study within the same individual female subjects exposed to different sets of male stimuli of the same type were also statistically consistent (see below).

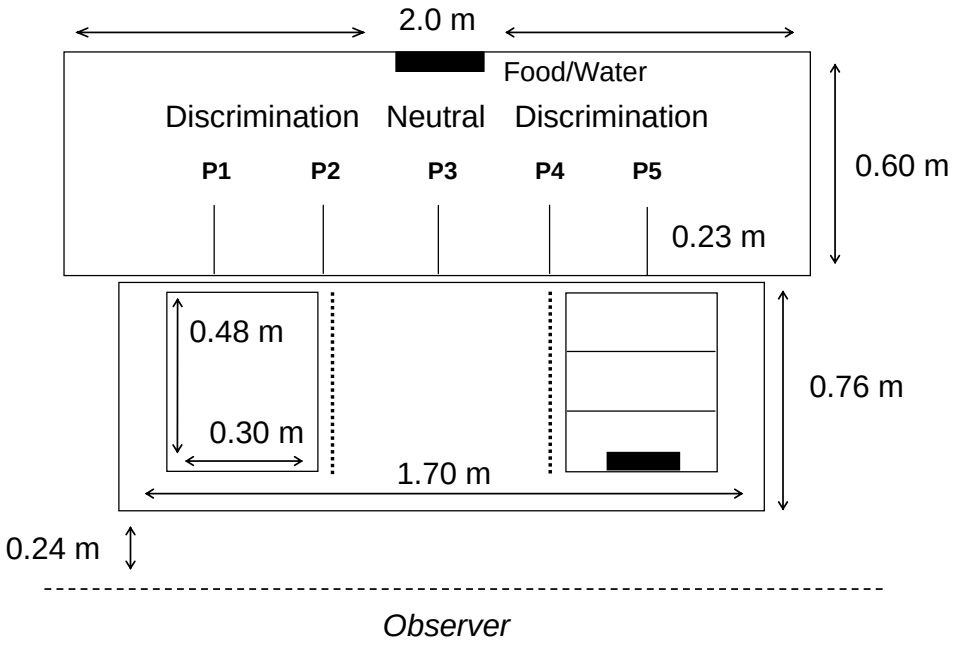


Figure 6.2: A representation of the test apparatus as viewed from above showing the female subject cage and male stimuli cages placed on either subject cage side. The division zones (P1, P2 [discrimination], P3 & P4, P5 [discrimination]) are indicated and placement of food and water in the neutral zone (subject cage) or at the back of the cage (stimuli cages) depicted. The dotted lines indicate the plastic divides that visually isolated the stimuli males from each other and the dashed line represents the screen behind which the observer was positioned.

Each female subject was initially placed in the test cage overnight to acclimatise and stimulus males were housed in identical cages to their experimental ones so as to be familiarised with the layout. All females were presented with the three different male stimulus type combinations. The wild-type colour morph and normal-reared songs and calls were quantified as the predicted 'preferred' visual (V) and/or acoustic (A) male features for each combination (Burley & Coopersmith 1987; Clayton & Pröve 1989; Swaddle & Cuthill 1994; Naguib & Nemitz 2007) so that the first combination (see below) presented two males that each only had one of the preferred features.

1. White normal-reared (A) over Wild-type Cross-fostered (V)
2. Wild-type normal-reared (V+A) over Wild-type Cross-fostered (V)
3. Wild-type normal-reared (V+A) over White normal-reared (A)

Trials were conducted in sets by introducing subject and stimuli into cages with screens present and leaving for 15 min. Screens were raised and a trial was begun followed by a 15 min break. Screens were lowered for swapping stimulus males between cages, with screens then raised for the third trial, followed by a 15 min break before the final fourth trial was run, this comprised one trial set. Stimulus males were replaced and the procedure repeated, with 8 trials only (two stimulus pairs) conducted in sequence for each female. Each female was exposed to two (of three) different individuals (across two trial sets) from each stimulus type for the three combinations (see above) with presentation order, subjects and stimulus individuals and cage side randomised and counterbalanced. Each female was observed over 24 trials each, 360 trials conducted in total for the experiment. Paired t-tests showed no difference in female discrimination between the two trial sets for each stimulus type combination (*White over Cross-fostered* t_{14} = 0.51, P = 0.62; *Wild-type over Cross-fostered* t_{14} = -1.65, P = 0.12; *Wild-type over White* t_{14} = 0.67, P = 0.51). For each trial the location of the female and calling and singing behaviour of the stimuli males was recorded on a 10-s scan sampling design. Vocalisation behaviour of the males was recorded to assess its possible influence on female spatial preferences (Houtman 1992).

Data were summarised to provide the average total proportion of 'discrimination time' (excluding time spent in the neutral zone) that each female spent with a particular male stimulus type for each combination (as indicated above) across the two trial sets. Data were also summarised to provide the average proportion of total time that subjects spent in the discrimination zones, an indication of the time they spent choosing between stimuli males (over residing in the neutral zone). Generalised Linear Mixed Model (GLMM) analysis showed no effect of trial order (across the 8 trials for each stimulus type) on total proportion of female discrimination time (*White over Cross-fostered* $F_{7,98}$ = 1.25, P = 0.28; *Wild-type over Cross-fostered* $F_{7,98}$ = 0.77, P = 0.61 and *Wild-type over White* $F_{7,98}$ = 0.56, P = 0.79). The averages of the total proportion of the time the male stimulus types spent calling and singing were also separately summed across the two trial sets against each individual female subject.

6.2.3 Statistical analyses

Parametric tests were applied to all raw female discrimination data (Shapiro-Wilk test for normality, all $W \geq 0.90$, all $P \geq 0.11$) and log-transformed ($\log_{10}(X+1)$) (Shapiro-Wilk test for normality: white males' calling behaviour $W = 0.96$, $P = 0.29$, all other males' calling and singing behaviour $W \geq 0.35$, $P \geq 0.0001$) male vocalisation data to assess significance ($\alpha < 0.05$) using Statview 5.0.1 and JMP 7 (Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA). Female discrimination data was log-transformed for data equality in the regression analyses.

One-sample t-tests with a random expectation of 0.5 (50%) were carried out on the average proportions of spatial discrimination for each of the three stimulus type combinations followed by GLMM analysis to assess differences in spatial discrimination between the three stimulus type combinations, with subject identity included as a random effect. I also used one-sample t-tests to compare the discrimination behaviour towards the three stimulus types with respect to spatial discrimination data for live wild-type conspecific male preference over live Bengalese finch males from previous research of mine (average proportion conspecific preference: 0.68, data from Chapter 3). This prior data showed the degree of preference for conspecific males over stimulus males fully displaying both visual and acoustic species-specific cues. A *post hoc* Student's t-test was applied to the least squares means of the spatial discrimination responses for the three stimulus type combinations (this study). I predicted, following published research (Burley & Coopersmith 1987; Clayton & Pröve 1989; Swaddle & Cuthill 1994; Naguib & Nemitz 2007), that the females would prefer the wild-type normal-reared males and that the disassociation of visual and acoustic cues would decrease discrimination strength.

I also carried out one-sample t-tests with random expectation of 0.8 (80% - each perch holds a random expectation of 20%) on the average proportion of total time female subjects spent in the discrimination zones for each of the three stimulus type combinations, followed by GLMM to assess differences in discrimination between stimulus type combinations, with subject identity included as a random effect. A *post hoc* Student's t-test was applied to the least squares means of the spatial discrimination responses of the three stimulus type combinations.

GLMM analysis was applied to the three male stimulus types' average calling and singing behaviour to assess differences between types and multiple regressions carried out against female discrimination for the three stimulus type combinations to determine the relationship between female preferences and male vocalisation behaviour.

For figures the untransformed values of individual females' spatial discrimination metrics are plotted, averaged across all trials in response to each stimulus type combination.

6.3 Results

As predicted, the one-sample t-test did not reveal significant spatial discrimination above random expectations for the stimulus type combination of *White over Cross-fostered* (t_{14} = 0.34, P = 0.74) and the females significantly preferred the Wild-type stimulus type in the *Wild-type over Cross-fostered* (t_{14} = 4.82, P = 0.0003) and *Wild-type over White* (t_{14} = 3.15, p = 0.0071) combinations (Fig. 6.3). The GLMM analyses confirmed these differences in spatial discrimination ($F_{2,28}$ = 6.11, P = 0.0063) with the *White over Cross-fostered* (*Least squares means [LSM]* estimate: 0.52 $\pm$ SE: 0.042) stimulus type combination differing significantly from the other two combinations (*Wild-type over Cross-fostered*: *LSM* 0.69 $\pm$ 0.042; *Wild-type over White*: *LSM* 0.62 $\pm$ 0.042*)* (Fig. 6.3). Only the *White over Cross-fostered* stimulus type combination showed significant difference from the comparative Wild-type over Bengalese finch data (t_{14} = -0.36, P = 0.0034) [*Wild-type over Cross-fostered* t_{14} = 0.35, P = 0.74; *Wild-type over White* t_{14} = -1.45, P = 0.17] (Fig. 6.3).

Similar to the above results, the one-sample t-tests did not show significant total discrimination time above random expectations for the stimulus type combination *White over Cross-fostered* (t_{14} = 2.00, P = 0.07), but the females significantly preferred to associate with the stimuli in the *Wild-type over Cross-fostered* (t_{14} = 4.45, P = 0.0005) and *Wild-type over White* (t_{14} = 7.86, P = 0.0001) combinations (Fig. 6.4). The GLMM analysis however did not show any significant differences in total discrimination time between the three stimulus type combinations ($F_{2,28}$ = 1.47, P = 0.25), *White over Cross-fostered* (*Least squares means* estimate: 0.86 $\pm$ SE: 0.021), *Wild-type over Cross-fostered*: *LSM* 0.88 $\pm$ 0.021; *Wild-type over White*: *LSM* 0.91 $\pm$ 0.021*)* (Fig. 6.4).

The average rates of males' calling behaviour significantly differed between stimulus types ($F_{2,73}$ = 6.47, P < 0.0026] with Wild-type males (*LSM* 0.19 $\pm$ 0.0072) calling less than both White (*LSM* 0.22 $\pm$ 0.0072) and Cross-fostered males (*LSM* 0.22 $\pm$ 0.0072) (Fig. 6.5a). The proportion of singing also significantly differed between all types ($F_{2,73}$ = 62.04, P < 0.0001) with Wild-type males singing the most (*LSM* 0.041 $\pm$ 0.0025) and Cross-fostered males singing the least (*LSM* 0.00018 $\pm$ 0.0025) (White males: *LSM* 0.012 $\pm$ 0.0025) (Fig. 6.5b). There was a significant interaction between female discrimination for the *Wild-type over Cross-fostered* stimulus type combination and wild-type males' calling behaviour (R^2 = 0.55, P = 0.0042) but all other interactions were not significant (all $R^2 \leq 0.25$, all $P \geq 0.18$).

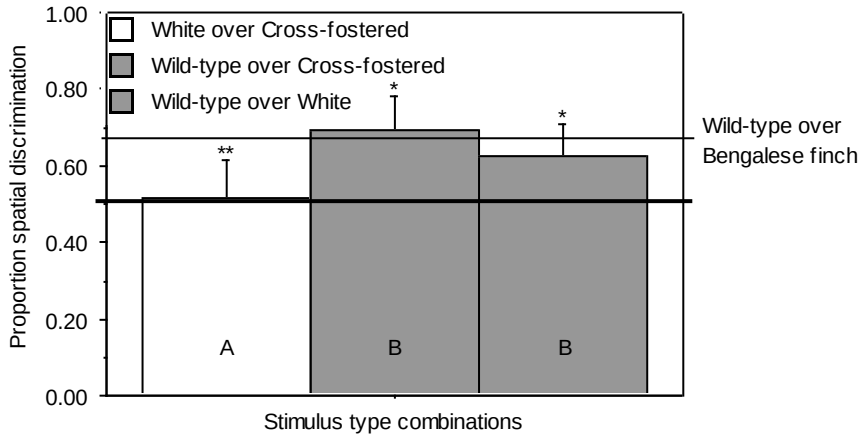


Figure 6.3: Spatial discrimination of female subjects (n = 15) in the presence of each male stimulus type combination. Mean of the raw values + upper 95% CI depicted where the **bold** solid line indicates random choice of 50% and single *asterisk* depicts significant deviation from this random expectation. The thin solid line indicates preference for wild type conspecific males over Bengalese finch heterospecific males as stimuli (from Chapter 3), double *asterisk* depicts significant deviation from this expectation. Stimulus type combinations with the same letter showed no significant *post hoc* differences.

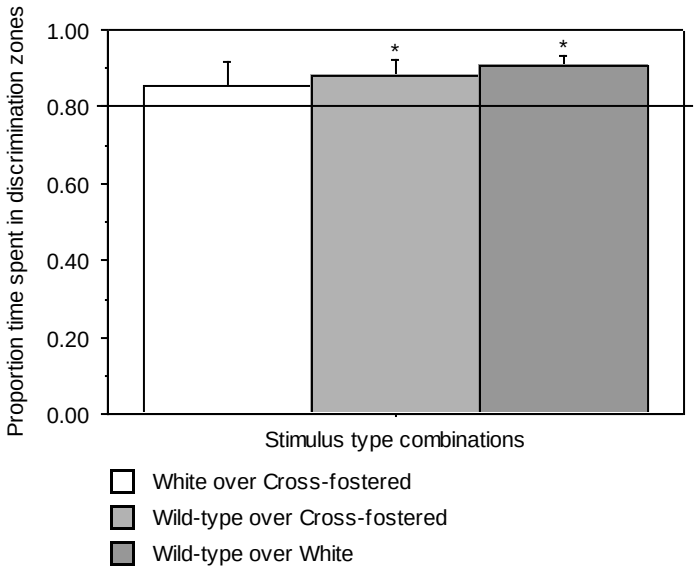


Figure 6.4: Proportion of total time spent in the discrimination zones of female subjects (n = 15) in the presence of each male stimulus type combination. Mean of the raw values + upper 95% CI interval depicted where the solid line equals random choice of 80% (each perch = 20% of total time) and *asterisk* depicts significant deviation from this random expectation.

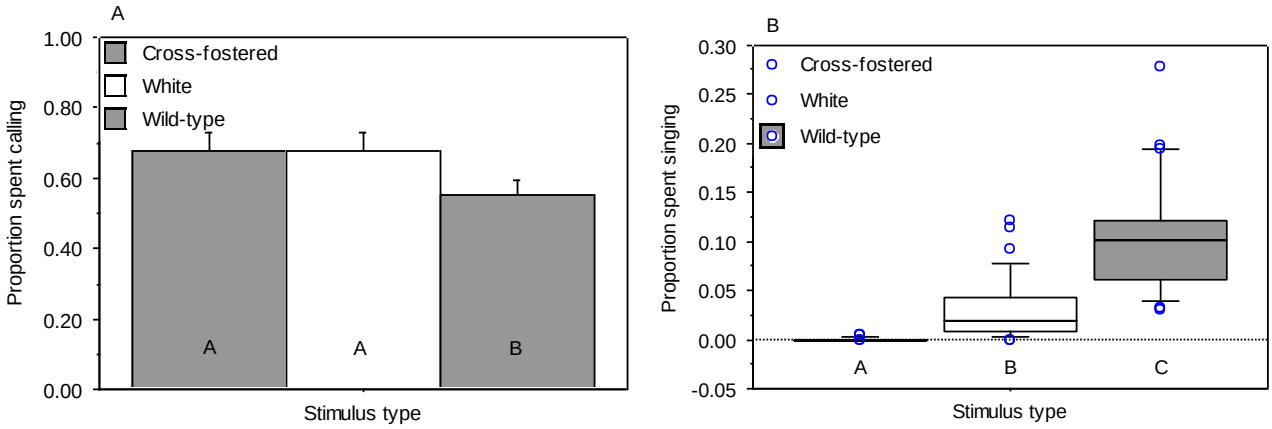


Figure 6.5a, b: The average proportion of time spent calling (a) and singing (b) for the three male stimulus types. Mean of the raw values + upper 95 % CI depicted and stimulus types with the same letter showed no significant *post hoc* differences.

6.4 Discussion

The results from this experiment support the salience of both visual and acoustic cues for female zebra finches in a social discrimination process when exposed to live stimulus males. Specifically, I found preferential spatial association with the wild-type normal-reared males which displayed the conspecific visual and acoustic features together. The wild-type colour morph displays ornamental, sexually selected traits in the chest band, cheek patches and red beak; these are all traits that are suggested to indicate physiological and developmental nutritional quality of the male through honest signalling for female mate choice (McGraw et al. 2003; McGraw 2007; Naguib & Nemitz 2007). The importance of wild type conspecific visual features was confirmed by the consistent association with males of the wild-type over the white morph with the black beak, when acoustic features of both morphs were similar (learned from conspecific, genetic fathers). Preference for some aspects of male conspecific visual traits in my experiments may have been based on self-referent phenotype matching using shared female and male conspecific traits (Hauber & Sherman 2001), or sexual imprinting by subject females on wild type parents (Vos et al. 1993 but see Walter 1973). Further studies using white morph female subjects and wild type females raised by white morph parents would extend these findings. Because I painted the beaks of white-morphed

males black to depart from acceptable adult conspecific traits (ten Cate et al. 2006), this non-conspecific (foreign) or juvenile trait likely meant that the female subjects did not recognise the white morph as a conspecific adult and previous evidence for the role of visual ornamentation in the con/heterospecific discrimination by zebra finches is to date limited (Brazas & Shimizu 2002). Specifically, my findings are statistically similar to spatial discrimination data from a previous study in which I used male Bengalese finches as foreign (heterospecific) stimuli over wild-morph normal-reared conspecific males (Fig. 6.3). Discrimination tests using both wild-type and white morphs of zebra finches over heterospecific male stimuli are nonetheless needed to confirm this scenario in future experimentation.

The importance of acoustic cues for conspecific recognition was confirmed by female zebra finches' consistent association with normal-reared over cross-fostered vocalisations of wild-type males (i.e., when visual cues were equivalent). This stimulus-context was also the instance in which there was a significant relationship between wild-type male vocalisations and female preference for those wild-type males, indicating the salience that acoustic cues represent during this discriminatory decision. In zebra finches, male songs and calls are critical features for female mate selection (Houtman 1992; Lauay et al. 2004), recognition of pair-bonded mates (Miller 1979a; Vignal et al. 2008), and species recognition (Clayton & Pröve 1989; Clayton 1990). Calling is particularly important for maintaining acoustic contact between individuals when visual contact is lost in their natural habitat while songs are particularly important for courtship and breeding (Zann 1996; Tchernichovski et al. 1998). These preferences can be innate or develop via imprinting on (foster) parents' songs (Miller 1979b; Lauay et al. 2004), but in either scenario they are reliable indicators of suitable mate bond (Holveck & Riebel 2007). Although the cross-fostered males rarely sang (similar to the low song-rate of their foster species, the Bengalese finch: ten Cate 1982), these males produced comparably numerous contact calls (at rates similar to normal-reared males: Fig. 6.5a). These calls, some of which are learned during ontogeny (Zann 1985) may have been sufficiently dissimilar from the calls of normal-reared males for female discriminations to occur when only acoustic cues differed between the stimuli. Alternatively, the low song rate and/or other, unmeasured species-typical visual and acoustic displays produced by the cross-fostered males may have limited the conspecific sexual cues available for female zebra finch subjects that resulted in their consistent spatial association decisions with normal-reared wild type male conspecifics. In turn, spatial proximity and affiliation is an important social

behaviour for zebra finches which are colonially living, foraging and breeding, species (Zann 1996). The lack of male vocalisation behaviour affecting more of the female choice decisions, despite significant male differences, is consistent with the documented independence from stimulus behaviour in female zebra finches' social association decisions (e.g., Forstmeier 2004, 2007; Rutstein et al. 2007). I cannot, however, conclusively state the motivational cause of captive-bred female zebra finches' spatial choices documented here, as sexual motivation or physiological state (Tchernichovski et al. 1998) and eventual pair bonding (ten Cate 1985) must be confirmed in a free-choice aviary paradigm with wild-caught birds (Rutstein et al. 2007). These free-flight studies with increased contact time between the females and males would also increase the female exposure to cross-fostered song and enable more conclusive determination of its influence on preferences.

The experimental mismatching of 'preferred' (i.e., conspecific) and foreign (i.e., heterospecific or novel) visual and acoustic features in this study, in accordance with previous findings (Galoch & Bischof 2006), elicited no preferential spatial affiliation by female subjects. If conspecific over foreign acoustic cues alone were sufficient for preference decisions (Holveck & Riebel 2007), then I might have seen a directional preference for the white normal-reared male but the lack of clear discrimination in my data indicates both visual and acoustic cues are combined for behaviourally clear-cut discriminatory decisions. Alternatively, as discussed above, females may have recognised the combination of conspecific songs with some aspects of the white morph's zebra finch-like visual displays (i.e., body size, shape, foot colour, cryptic UV signals: Bennett, Cuthill, Partridge & Maier 1996) and the conspecific visual displays with the zebra finch-like temporal patterning of the cross-fostered males' vocalisations (Clayton 1989) as sufficiently conspecific-like, leading to the documented lack of spatial preference between these stimulus types. Future studies using model-presentations and/or video presentations with acoustic playbacks are needed to evaluate these alternatives.

I conclude that these new data on the spatial discrimination of live conspecific males, of different visual and acoustic morphs, by female zebra finches suggest social choice using conspecific cues of both visual and acoustic sensory modalities combined. The relative roles of cues displayed by different male colour morphs and normal or cross-fostered vocalisations in the eventual pair formation decisions by female zebra finches are avenues for future research.

7

Conspecific-only experience during development reduces the strength of heterospecific song discrimination in zebra finches (Taeniopygia guttata): a test of the optimal acceptance threshold hypothesis

7.1 Introduction

Auditory perception is critical for correct species identification across avian lineages (Kroodsma & Miller 1996). The zebra finch (*Taeniopygia guttata*) is both a behaviourally (Zann 1996) and neuroanatomically (Wade & Arnold 2004) sexually dimorphic species and model study system for song production and song perception research (Riebel 2003). In zebra finches only males sing and the song is typically used for species recognition (Clayton 1990), to attract females as mates (Clayton & Pröve 1989), and in male-male communication (Riebel et al. 2002). Both female and male zebra finches learn to prefer songs of familiar individuals during a sensitive phase (35-65 days posthatch) of development (Eales 1985, 1987b; Clayton 1988a), including the males' ability to learn to sing heterospecific-like songs when cross-fostered by, for instance, Bengalese finches (*Lonchura striata* vars. *domestica*) (Eales 1987a; Clayton 1989).

However, both song production and song perception also include experience-independent components: tutor-deprived males produce a rudimentary version of conspecific song (Eales 1985, 1987b), while song-deprived males and females both show behavioural and neurophysiological preferences for conspecific songs over heterospecific songs (Braaten & Reynolds 1999; Lauay et al. 2004; Hauber et al. 2007a). Behavioural and physiological evidence also suggests there are at least two ontogenetic routes of experience-dependent song perception learning in zebra finches. These include males' experience with (i) own songs (Pytte & Suthers 1999) and females' and males' experience with (ii) other males' songs (Cynx 1993; Riebel et al. 2002; Hauber et al. 2007b). When given a choice between learning from conspecifics or heterospecifics, male zebra finches preferentially learn to copy songs of conspecifics (Immelmann 1969; Eales 1987a), but when given a choice between learning from conspecifics singing zebra finch songs and cross-fostered conspecifics singing Bengalese finch-like songs, naïve male zebra finches learn songs from both tutor types indiscriminately (Clayton 1988b).

Bengalese finches are a domesticated strain of a related estrildid finch and sing a typically longer song in the same frequency range as zebra finches, but with more repeated elements and fewer harmonics per element (Clayton 1989). Cross-fostered zebra finches, raised by Bengalese finch parents, will partially learn their foster father's song, producing an intermediate song which consists of Bengalese finch elements in a shortened zebra finch

phrase temporal structure (Eales 1987a; Clayton 1989). By examining behavioural responses to species-typical and cross-fostered song playbacks we can further understand the auditory perceptual processes, and critically identify the acoustic features of conspecific songs that birds perceive as salient for enabling correct species identification (Emlen 1972; Brenowitz 1983). In our experiments we examined the behavioural responses of zebra finches to normal, cross-fostered, and Bengalese finch songs to determine whether *female and male normal-reared zebra finches equally prefer conspecific songs over cross-fostered and Bengalese finch songs.*

In most research to date zebra finches for behavioural experimentation typically originate from local breeders or are bred in controlled indoor institutional aviaries housing a single species of subjects (e.g., Hauber et al. 2007a, b). But when investigating auditory discrimination and song preferences in a laboratory situation it is necessary to consider what impacts rearing conditions that differ in social and environmental parameters may have on subsequent behavioural preferences. Even though captive-bred zebra finches show an innate bias for conspecific song over unrelated heterospecific song playbacks in the absence of early exposure to singing male conspecifics (Braaten & Reynolds 1999; Lauay et al. 2004), a conspecific rearing environment during the sensitive period is also important for males' to produce species-typical songs (Eales 1987a; Volman & Khanna 1995; Tchernichovski & Nottebohm 1998) and for females and males' conspecific preference in mate choice (Neubauer 1999; Pytte & Suthers 1999; Lauay et al. 2004). Additionally, environmental enrichment is important for response to novelty during any protocol used in laboratory testing (e.g., Meehan & Mench 2002). But it is still unclear what effects there might be of equally enriched captive housing setups exposed to constant artificial environmental parameters indoors (i.e., regimented light schedules and predictable airflow) versus exposed to variable weather conditions outdoors on behavioural responses in social choice tests.

Optimal acceptance threshold theory (Reeve 1989) is an integrative model of recognition systems that explains the perceptual and behavioural bases of conspecific recognition decisions in the light of ultimate fitness benefits. This theory has been frequently applied to discrimination of potentially harmful social partners, including non-nestmates in social insects (Starks et al. 1998) and brood parasitic eggs in host birds (Hauber et al. 2006; Moskát & Hauber 2007). But does optimal acceptance theory apply to conspecific recognition in song learning birds from different early social environments? The theory predicts that

individuals exposed to conspecifics only during development would have a less stringent acceptance threshold because they do not encounter unsuitable social partners during development. In contrast, individuals exposed to a variety of potential social partners would have a more restrictive acceptance threshold to discriminate between suitable (i.e., conspecific) and unsuitable (i.e., heterospecific) partners (Fig. 7.1).

Although research to date has considered the effects of social isolation from conspecifics on subsequent behavioural and neurophysiological song discrimination (Neubauer 1999; Pytte & Suthers 1999; Riebel 2000; Lauay et al. 2004; Hauber et al. 2007a) or on species preference using con- and heterospecific songs (Braaten & Reynolds 1999; Lauay et al. 2004), there is no examination of the specific effect of social experience with conspecifics only on song discriminatory preferences in zebra finches. In turn, the effect of environmental enrichment and early experience on response to novelty has been examined in captivity (e.g., Fox & Millam 2004, 2007) but given the potential differences in social behaviours of captive and wild-caught zebra finches (Rutstein et al. 2007) and its role as a major model system for neuroethological research on song recognition (Chapter 2; Hauber et al. 2007b), it needs to be examined what the influence of conspecific-only social experience might be on the behavioural responses of captive zebra finches regarding conspecific discrimination. Development without any heterospecific stimulation and constant environmental conditions are atypical for the zebra finches' natural environment (Zann 1996). Therefore, here we specifically consider whether in captivity, *the rearing conditions during development have an effect on song discrimination in female versus male zebra finches in response to songs of normal zebra finches, songs of zebra finches cross fostered by Bengalese finches and songs of Bengalese finches*. Specifically, we also evaluate the prediction of evolutionary theory which predict more restrictive species recognition templates and preferences for the choosier, female sex (Jennions & Petrie 1997, Chapter 4).

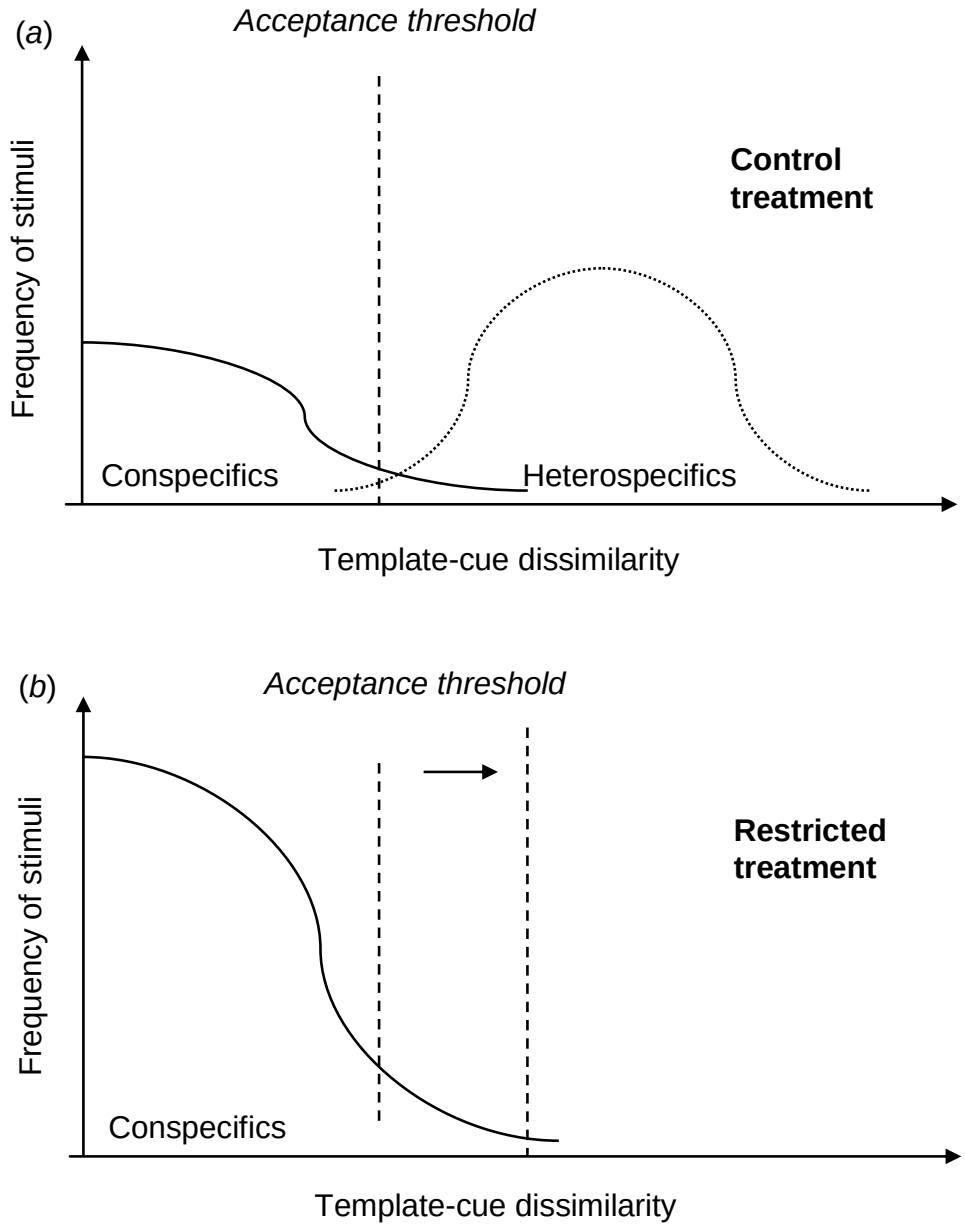


Figure 7.1: A graphical representation of the shift towards a more permissive acceptance threshold in the context of song discriminatory abilities dependent on acoustic environment during ontogeny: Control subject treatment (*a*), Restricted subject treatment (*b*). Adapted from Reeve (1989).

7.2 Methods

7.2.1 Study subjects and housing

For our experiments we used 14 female and 15 male zebra finches that had been raised by their genetic parents in our outdoor aviaries during 2006-2007 with breeding occurring all year round. The subjects' physical contact was limited to conspecifics within their aviaries but they were exposed to a sensory environment that included naturally occurring wild bird species in suburban Auckland, New Zealand (most common surrounding species: blackbird *Turdus merula*; house sparrow *Passer domesticus*; song thrush *Turdus philomelos*; starling *Sturnus vulgaris*; Tui *Prosthermadera novaeseelandiae*), in an outdoor setting, including local weather conditions.

For a comparison we reared 17 female and 17 male zebra finches in a controlled indoor single species aviary on the University of Auckland City Campus in isolation from the outdoors, with fluorescent photoperiod that mimicked that of the outdoor aviary, including a compact Arcadia^{TM} fluorescent bird lamp 2.4 %UVB and 12 %UVA, mean temperature 21$\pm$ 3°C, constant airflow and daily humidity 35-65%. These subjects were reared by their genetic parents and socially and acoustically exposed to conspecifics only until the time of experimentation and comprised our Restricted subject group.

All subjects were sexually mature (100 days – 1 year old, Zann 1996), naïve to auditory playback experimentation and captive-bred from domesticated stock due to an import embargo on wild bird species into New Zealand. Subjects were fed *ad libitum* a commercial finch dry-seed mix (Animates^{TM}) with water replenished daily. Dietary supplements were provided weekly (see Chapter 3) and all experiments approved by the University of Auckland Animal Ethics Committee.

7.2.2 Preparation of auditory playbacks

Unfamiliar songs from males of 6 zebra finches, 6 Bengalese finches, and 6 cross-fostered zebra finches were used for song playbacks. The songs of 3 individuals of each stimulus type were recorded in a sound attenuated chamber and digitised at 32 kHz (TDT Technologies^{TM})

(courtesy S. Woolley and F. Theunissen). The remaining songs for each stimulus type were recorded in a sound attenuated chamber using a Marantz™ PMD 671 solid state recorder with a Sennheiser™ ME66 shotgun microphone, recording the males' song at a sample rate of 44.1 kHz and 16-bit resolution, digitising it directly as a wave file.

Playbacks were created in Raven Sound Analysis Software v.1.2.1 (Charif et al. 2004). All songs were bandpass filtered 500-15000 Hz and amplitudes were root mean square equalised. All songs consisted of 1-4 motifs including introductory notes, repeated at approximately 10 s intervals for the 10-min playbacks (see below).

7.2.3 Experimental set-up

Sequential song playback trials (Hauber et al. 2001) were conducted on the university campus during August 2007 - April 2008. Preliminary multivariate analyses showed no effect of month of testing on the different behavioural measures (all $P \geq 0.60$) (Cynx & Nottebohm 1992). The room was fluorescently lit with an ambient temperature of 20 ± 2°C. The testing apparatus consisted of a single elongated cage (length 2.0 m, width 0.6 m and height 0.8 m) placed at 0.87 m above the ground that was divided with 5 perches labeled P1-5 where P1 and P2 and P4 and P5 near each side (and the immediate surrounding ground area) were classed as side-proximity or discrimination zones while P3 in the centre (including immediate ground area) was classed as a central neutral zone (Fig. 7.2). A table clothed in white fabric was placed directly in front of the test cage and two small Radioshack™ AMX 3 speakers were placed on a box in front of P1 or P5 (in random) also clothed in white fabric. A larger clothed box in front of the opposing end perch eliminated visual cues for associating with the auditory stimulus (Fig. 7.2). A Dell latitude D620 laptop located behind the observer's screen was used for all auditory playbacks that were delivered equally at ~ 70 dB SPL (measured at the centre of the two end perches by a digital sound level meter, Radioshack™ 33-2055).

Food and water *ad libitum* were placed in the neutral zone and the entire set-up was located behind a fabric screen with mesh holes to permit the observer to watch and record in real time, all trials unseen.

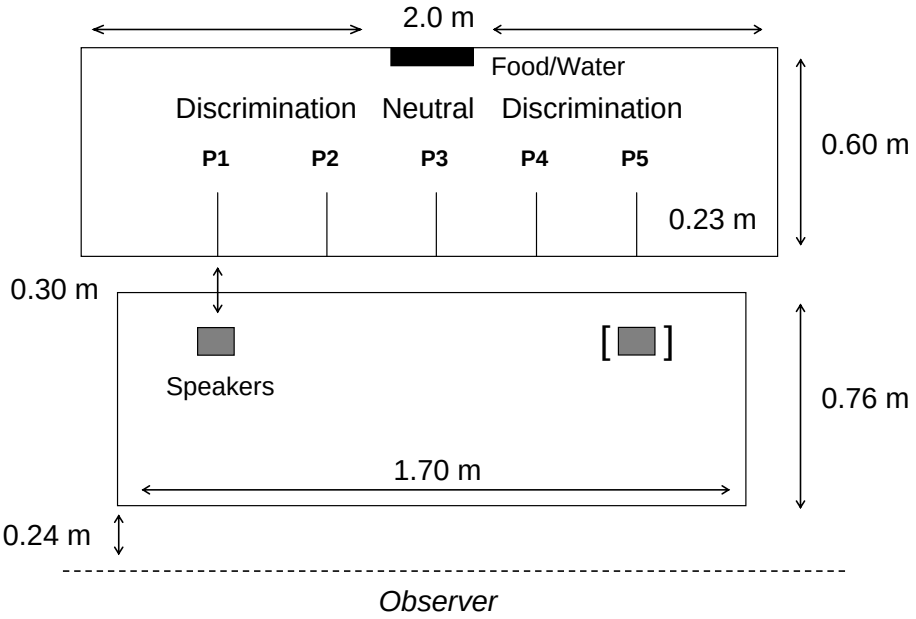


Figure 7.2: A representation of the test apparatus as viewed from above portraying the subject cage and playback speakers placed on either test cage side. The division zones (P1, P2 [discrimination], P3 & P4, P5 [discrimination]) are indicated and placement of food and water in the neutral zone depicted. The dashed line represents the screen behind which the observer was positioned.

7.2.4 Experimental procedure

All trials (duration: 10 min.) were run daily from 12 midday local time onwards to adhere to a standardised time schedule. We allowed 24 hr for each individual subject to become familiarised with the test cage 1-3 days prior to conducting the playback trials.

The subjects were removed after their acclimatisation period into their holding aviaries and then individually placed back into the test cage before trials, allowing 15 min. to adjust. Upon reintroduction into the test cage they would appear to remember the layout from their previous familiarisation period and immediately adapt to the surroundings (DLMC pers. obs.). This schedule enabled several subjects to be tested during a single day as only four consecutive playback trials were run with each subject per day to minimise the possible effects of lack of motivation on behavioural responses.

Responses by female and male Control and Restricted zebra finches to song playbacks were quantified through multiple behavioural measures. These consisted of (1) spatial association (proximity to speakers), a frequently used discrimination metric (reviewed in Forstmeier & Birkhead 2004), (2) wing-flicks, which are produced in response to a stimulus and can indicate curiosity, fear, agonistic or sexual interest (Goodwin 1982) (3) 'distance' calls, produced in contexts such as sudden excitement (Zann 1996) (4) 'tet' calls, which are soft contact calls (Zann 1996), and for males only (5) song bouts, which are frequently produced in wild flocking contexts (Dunn & Zann 1996). We also measured overall subject response as defined by $\geq$ 1 min duration of any movement (i.e., preceding movement, the subjects were typically frozen, seemingly induced by novel stimuli: Stripling et al. 2003). A response was scored as a 'Yes' or 'No' for each 10 min trial depending on whether movement away from the initial perch occurred. Trials with no movement were included in behavioural analyses (i.e., scores of zero) but subjects that did not move (respond) across all 12 trials were excluded from any analyses. We obtained responses from only 12 females and 12 males for both Control and Restricted subjects (i.e., n = 5 Control and n = 10 Restricted subjects did not move), but this difference in subject response was not significant between the two treatment groups (Fisher's Exact Test $P = 0.38$).

During each trial, the perch location and behaviour (Goodwin 1982; Zann 1996) of the subject was recorded using a scan-sampling design every 10 s with all behaviours recorded by the same observer (DLMC). In addition, the total number of distance and tet calls and song bouts produced during each trial were counted. After the first trial the subject was left without a playback for a 15 min. break then the second trial commenced. A 4-trial sequence was run for the particular subject before being replaced with a new subject who was left for 15 min. prior to the commencement of their first trial. Two different song files were used for each stimulus type and presented once on both test cage sides where each subject was observed over sequential presentations of 4 trials per stimulus type. A total of 12 trials were run for each subject over the 3 stimulus song types (576 trials in total). Individual songs, stimulus type and test cage side presentation were all randomised and counterbalanced.

7.2.5 Statistical analyses

The data were collated to provide the proportion of all their trials across the three stimulus types combined that each individual Control and Restricted subject showed a response. The

data were then also summarised to provide (1) the proportion of total time that each subject spent in spatial proximity (included both P1 & P2 or P4 & P5) to the auditory stimulus (measured as a proportion of the 10 s samples, not measured in real time), (2) total proportion of time spent producing wing flicks, the total numbers of (3) distance calls, (4) tet calls, and (5) song bouts (males only) produced (in any area of the test cage), across trials with the same stimulus type. The data for each behavioural measure were averaged for each subject in response to each of the three stimulus types. This resulted in each subject contributing a single data point for each behavioural metric for each stimulus type. All proportional data were initially log transformed ($\log_{10}(X + 1)$), followed by parametric tests to assess significance ($\alpha < 0.05$) using Statview (5.0.1) and JMP 7 (Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA) for all analyses.

The effect of rearing conditions and sex on overall subject response, spatial discrimination, wing-flicks, distance calls and song bouts (rearing conditions only) for female and male Control and Restricted subjects were assessed separately using Generalised Linear Mixed Models (GLMM) with subject identity added as a random effect. One-way ANOVA analyses revealed no significant interaction between the effects of sex and rearing conditions (all $P \geq 0.06$) for the measures of spatial discrimination, wing-flicks, and distance calls, therefore, either sex or rearing conditions were added as random effects into the converse analyses. There was however, a significant interaction between sex and rearing conditions on the measure of tet calls ($P = 0.0004$). Tet calls were then analysed by inclusion of the effects of both sex and rearing conditions together. A *post hoc* Student's t-test was used on the least squares means estimates of the response variables for each behavioural measure.

One-sample t-tests were applied to assess deviation from the random expectation of 50% response rate for total response of female and male Control and Restricted subjects. They were also applied to assess deviation from the random expectation of 40%, log-transformed as 0.146 (i.e. random expectation of 20% for each of the 5 perches) for the transformed proportions of total discrimination time that the female and male and Control and Restricted subjects spent with each stimulus types' songs. Finally, a principal component analysis was applied to reduce variation across all behavioural response variables excluding the measurement of song bouts for which data was available for only half the subjects (males only).

Based on both evolutionary theory (Jennions & Petrie 2007) and the sexual dimorphism of zebra finch vocal and neuroanatomical ontogeny (Wade & Arnold 2004), it was predicted that there would be sex differences in the responses towards the presented stimuli. Females were expected to be more discriminative than males, although evidence from recent song playback research indicates these effects might be minimal (Riebel et al. 2002). It was also predicted that Control subjects would prefer the zebra finch songs but would differentiate between the cross-fostered and Bengalese finch songs (Clayton 1990; Clayton & Pröve 1989; Sturdy et al. 1999; Braaten et al. 2006). Finally, based on optimal acceptance theory (Reeve 1989) and the effects of differing rearing conditions on song development and behavioural responses (Neubauer 1999; Pytte & Suthers 1999; Riebel 2000; Riebel 2003; Lauay et al. 2004), it was predicted that Restricted subjects would show decreased discrimination between stimuli songs and overall decreased response to the novel stimuli.

7.3 Results

Overall, Control subjects responded significantly more to the song playbacks than the Restricted subjects across trials for all stimulus types combined ($F_{1,44}$ = 6.54, P = 0.014). There was a significant interaction between subject sex and rearing conditions ($F_{1,44}$ = 4.15, P = 0.05) whereby female Restricted subjects responded the least and were the only group that did not significantly differ in response rate from random expectations (Fig. 7.3).

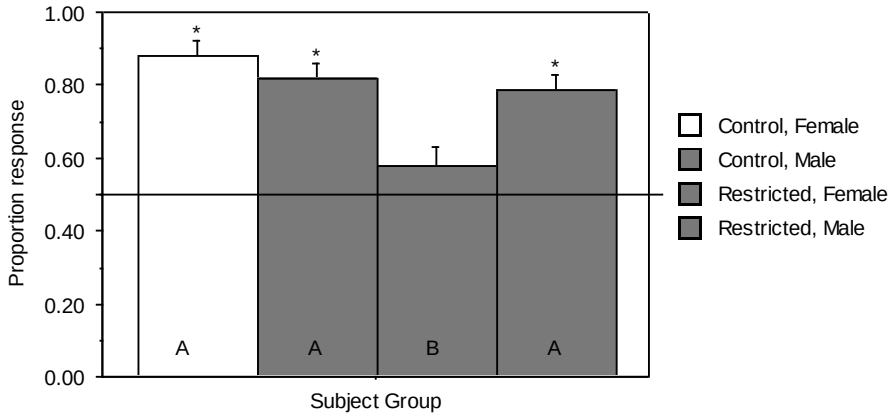


Figure 7.3: The average proportion of trials that female and male Control and Restricted subjects behaviourally responded. The solid line indicates a random response rate of 50%, *asterisk* depicts significant deviation from this expectation and subject groups that do not have the same letter showed significant *post hoc* differences.

7.3.1 Effect of subject sex on song discrimination

GLMM showed that there was a significant effect of sex on the spatial discrimination measure ($F_{4,94.29}$ = 4.19, P = 0.004) (Fig. 7.4a) with male subjects spending more time in proximity of the zebra finch songs over both Bengalese finch and cross-fostered songs in comparison to female subjects who spent more time in proximity of both zebra finch and cross-fostered songs over Bengalese finch songs (Fig. 7.4a). Additionally, the one sample t-tests for spatial discrimination revealed only the male responses to the zebra finch songs were significantly different (greater) from what would be predicted by random (40%) (Fig. 7.4a).

In contrast, there was no significant effect of sex on the proportion of time spent producing wing-flicks ($F_{4,90.7}$ = 1.88, P = 0.12) (Fig. 7.4b), although male subjects did discriminate between zebra finch and Bengalese finch songs. There was no significant effect of sex or on the number of distance calls produced ($F_{4,91.4}$ = 1.44, P = 0.23) (Fig. 7.4c).

Finally, although there was a significant interaction between the effects of sex and rearing conditions (P = 0.0004) on the number of tet calls produced there was no significant effect of both of these combined on this behavioural measure ($F_{8,88.3}$ = 1.46, P = 0.18) (Fig. 7.4d).

The data for female and male subjects were pooled for analyses on the effect of rearing conditions on song discrimination as measured by spatial discrimination, wing-flicks, distance calls and song bouts with sex added as a random effect (excluding song bouts).

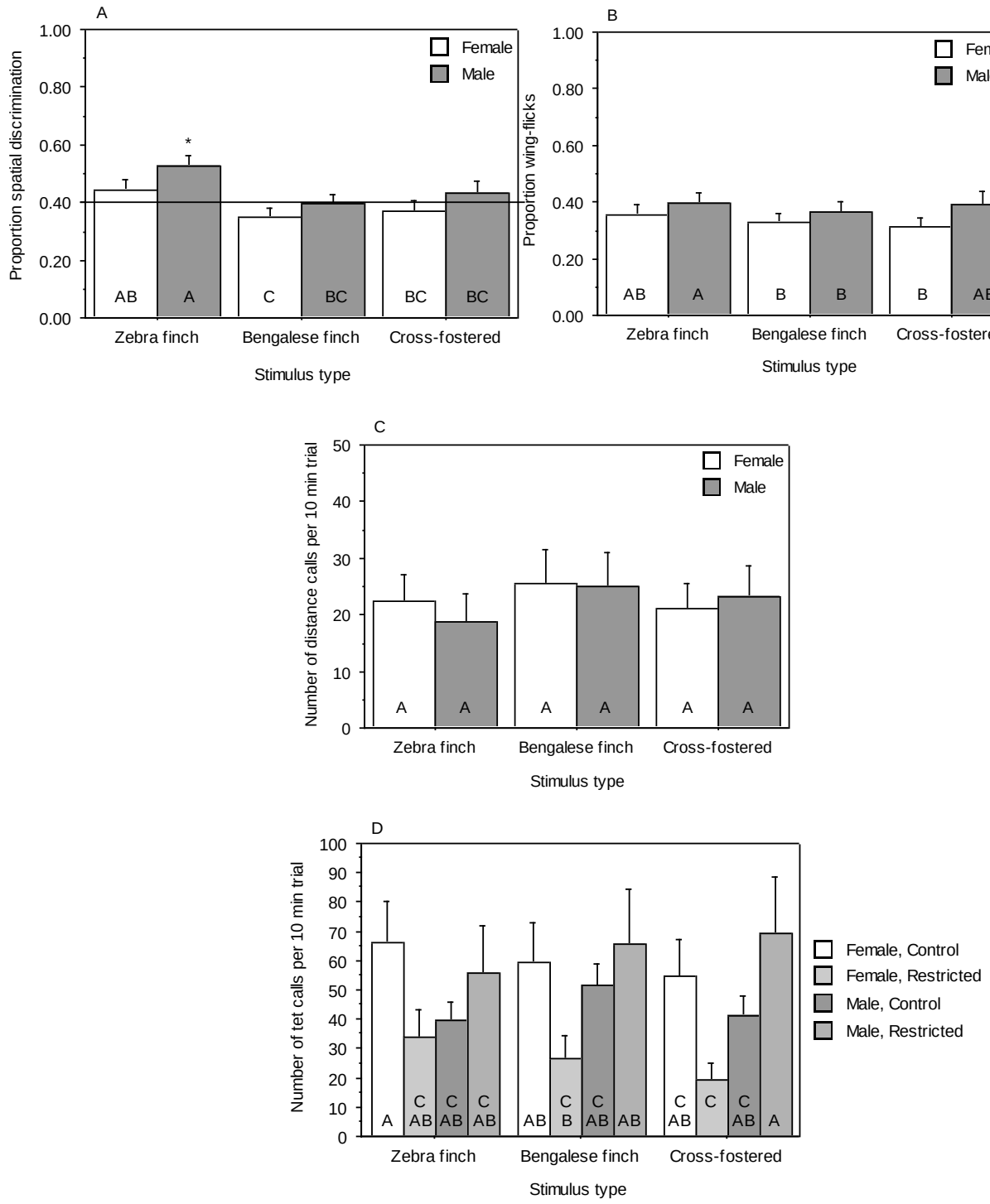


Figure 7.4a-d: The average proportions or numbers for female (n = 24) and male (n = 24) subjects for each response variable in the presence of each stimulus types' songs (a: spatial discrimination, b: wing-flicks, c: distance calls, d: tet calls [includes the effect of rearing conditions]). Mean of the raw values + SE depicted where solid line (a) indicates random choice of 40% and *asterisk* depicts significant deviation from this random expectation. Stimulus types in combination with dissimilar sex (and dissimilar rearing conditions for [d] tet calls) that do not have the same letter showed significant *post hoc* differences.

7.3.2 Effect of Rearing Conditions on Song Discrimination

Rearing conditions had a significant effect on the spatial discrimination responses towards each stimulus types' songs ($F_{4,94} = 5.54$, $P = 0.0005$) (Fig. 7.5a). Specifically, as predicted by conspecific acceptance threshold theory (Fig. 7.1), Control subjects spent more time in spatial proximity to the zebra finch songs over both Bengalese finch and cross-fostered songs but Restricted subjects showed no proximity variation between presented stimuli songs (Fig. 7.5a). Additionally, the one-sample t-tests revealed that only the Control subjects' spatial proximity responses towards the zebra finch songs showed association significantly different (greater) than random expectations (Fig. 7.5a).

There was also a significant effect of rearing conditions on the wing-flick responses towards each stimulus types' songs ($F_{4,90.52} = 2.86$, $P = 0.028$) (Fig. 7.5b). Specifically, Control subjects produced more wing-flicks in response to zebra finch songs over both Bengalese finch and cross-fostered songs whereas Restricted subjects exhibited similar responses to all presented stimuli songs (Fig. 7.5b).

There was no significant effect of rearing conditions on the responses towards each stimulus types' songs as measured by distance calls ($F_{4,91.63} = 2.08$, $P = 0.09$), although Control subjects did differentiate between zebra finch and Bengalese finch songs but not cross-fostered songs. Restricted subjects in contrast did not show any discrimination between stimulus types' songs (Fig. 7.5c). There was also (in this behavioural measure only) an overall effect of rearing conditions, irrespective of stimulus types' songs, on the number of distance calls produced with Control subjects calling significantly more than Restricted subjects ($F_{1,93.28} = 15.87$, $P = 0.0001$) (Fig. 7.5c). Finally, there was no significant effect of rearing conditions on the responses towards each stimulus types' songs as measured by song bouts ($F_{4,44} = 0.09$, $P = 0.98$) (Fig. 7.5d).

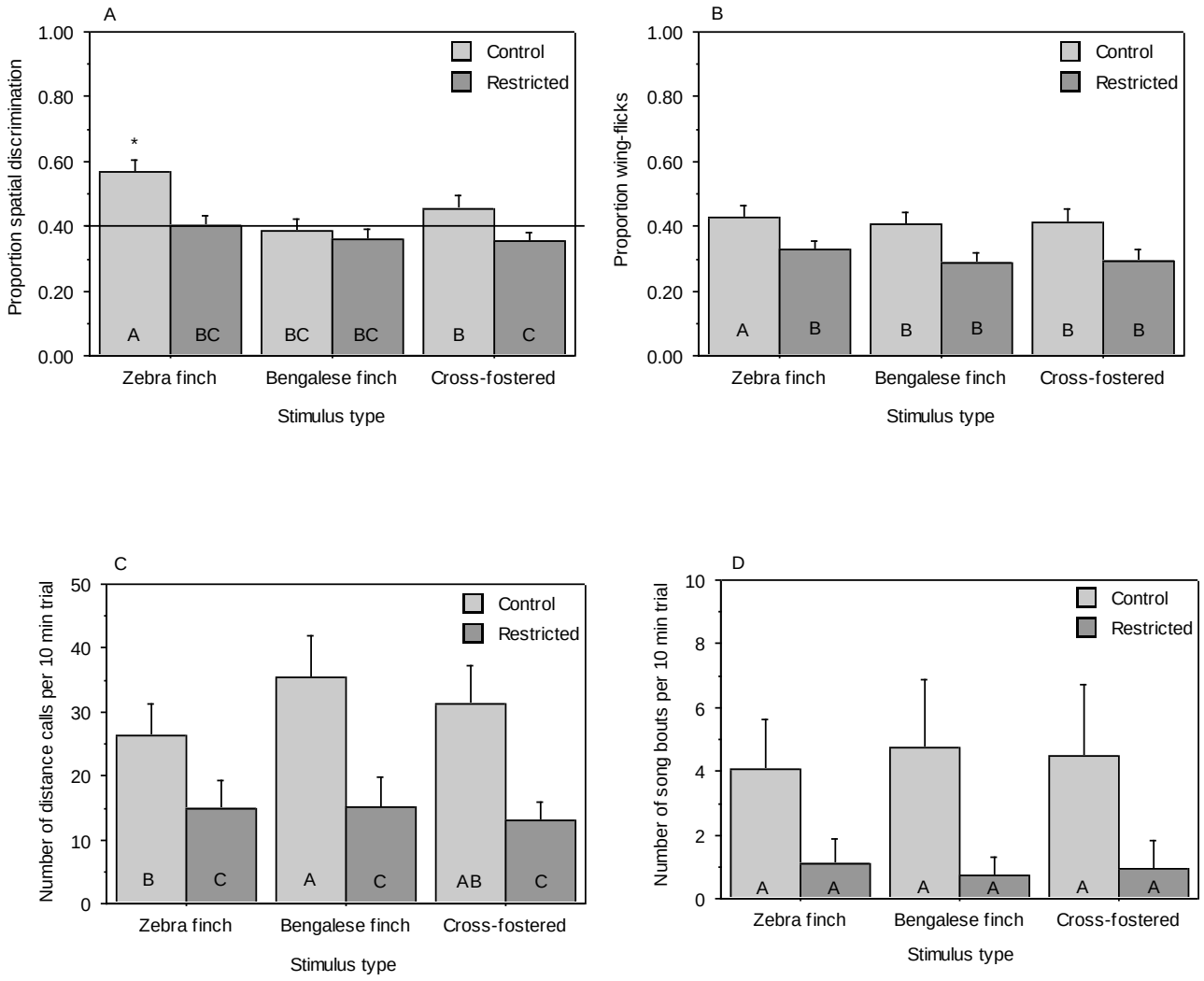


Figure 7.5a-d: The average proportions or numbers for Control (n = 24) and Restricted (n = 24) subjects for each response variable in the presence of each stimulus types' songs (a: spatial discrimination, b: wing-flicks, c: distance calls, d: song bouts). Mean of the raw values + SE depicted where solid line (a) indicates random choice of 40% and *asterisk* depicts significant deviation from this random expectation. Stimulus types in combination with subjects of different rearing conditions that do not have the same letter showed significant *post hoc* differences.

7.3.3 Principal component analysis

A principal component analysis was applied to reduce variation across all behavioural response variables excluding the measurement of song bouts for which data was available for only half the subjects (males only). The first principal component (PC1) explained 44% of the variation in the data (eigenvalue 1.77), and the second principal component explained 26% of the variation in the data (eigenvalue 1.05) accounting for 70% of the total variation (Table 7.1). All four response variables were positively associated with PC1 and PC2 except for the spatial discrimination metric which was negatively associated with PC2 (Table 7.1).

GLMM showed that there was no significant effect of rearing conditions on the behavioural responses towards each stimulus types' songs as measured by PC1 ($F_{4,92.83} = 1.23$, $P = 0.30$) (Fig. 7.6a) although Control subjects were all positively associated with PC1 and Restricted subjects were all negatively associated with PC1, a significant effect of rearing conditions overall, irrespective of stimulus types' songs ($F_{1,86.13} = 13.44$, $P = 0.0004$) (Fig. 7.6a).

There was in contrast, a significant effect of rearing conditions on the responses towards each stimulus types' songs as measured by PC2 ($F_{4,138} = 3.40$, $P = 0.0043$) with Control subjects showing equal negative association with PC2 in response to zebra finch and cross-fostered songs but were positively associated with PC2 in response to Bengalese finch songs (Fig. 7.6b). Restricted subjects however, showed no significant variation in their responses to each stimulus types' songs (Fig. 7.6b).

Table 7.1: PC1 and PC2 eigenvectors, eigenvalues and cumulative percentage variance from the principal component analysis on the measured behavioural response variables.

Behavioural response	**PC1**	**PC2**
Spatial discrimination	0.42	-0.52
Wing-flicks	0.65	0.054
Distance calls	0.60	0.031
Tet calls	0.20	0.85
Eigenvalues	1.77	1.05
Cumulative variance	44.15	70.46

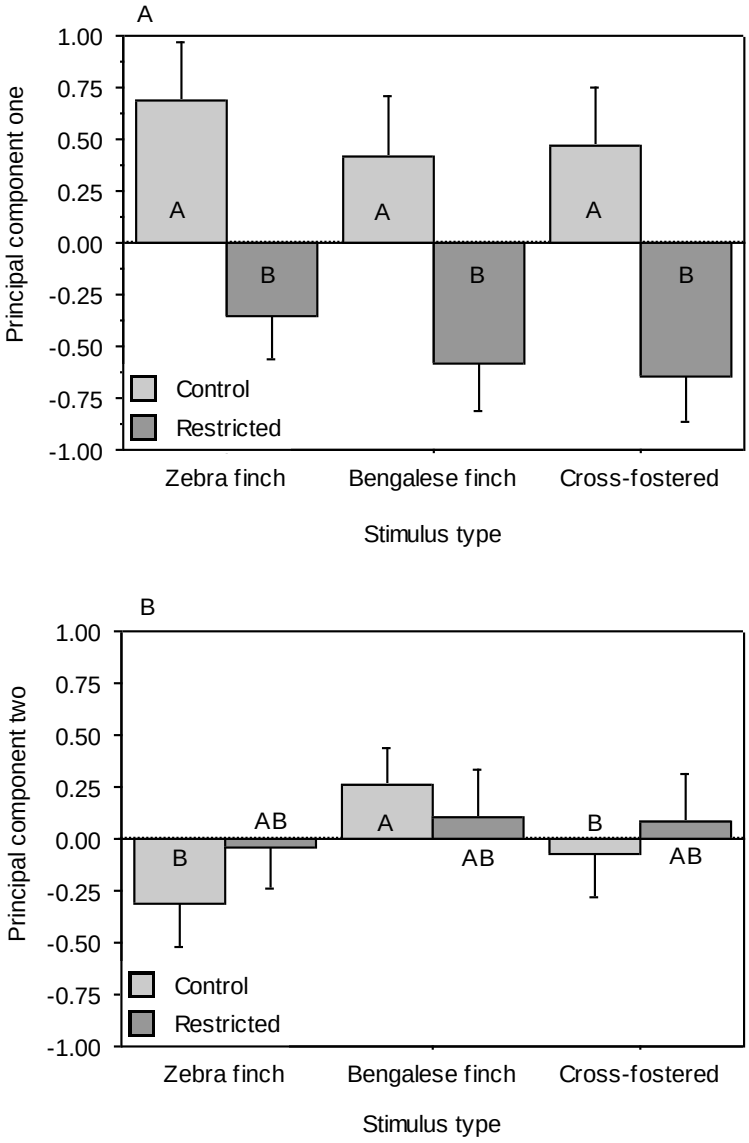


Figure 7.6a, b: The responses of Control (n = 24) and Restricted (n = 24) subjects in the presence of each stimulus types' songs in relation to principal component one (a) and principal component two (b). Mean of the principal component values + SE depicted. Species in combination with subjects from dissimilar rearing conditions that do not have the same letter showed significant *post hoc* differences.

7.4 Discussion

Behavioural measures by female and male zebra finches from two different rearing conditions revealed general similarities between the sexes in the discrimination between conspecific songs, heterospecific songs, and cross-fostered conspecifics singing heterospecific-like songs. These results of overall sex similarity are comparable to findings from females and males in previous song discrimination experiments (Braaten & Reynolds 1999; Riebel et al. 2002; Braaten et al. 2006; Chapter 8) indicating the shared importance and implying a shared ontogenetic pathway of song perception preference between the sexes. Consistent with optimal acceptance threshold theory (Reeve 1989) (Fig. 7.1), we documented reduced levels of behavioural discrimination in the Restricted treatment in comparison to Control zebra finches. Finally, and importantly, different behavioural measures revealed different strengths and patterns with respect to both the effects of sex and social ontogeny on the song discrimination responses across subjects (Rutstein et al. 2007).

In light of the vast sex differences in the neuroanatomical (Wade & Arnold 2004) and behavioural (Zann 1996) song systems of the zebra finch we might have expected distinct responses between female and male subjects but our findings were more consistent with previous research that has indicated a lack of sex effect in song discrimination playback experimentation (Braaten & Reynolds 1999; Riebel et al. 2002; Braaten et al. 2006; Hauber et al. 2007b). The only behavioural measure of discrimination that presented a significant sex difference was the measure of spatial discrimination. Here, contrary to our previous findings, the females did not discriminate between zebra finch songs and cross-fostered songs (Chapter 6). However, the significant sexual dimorphism documented in only one of our four behavioural metrics employed, has ambiguous implications regarding other behavioural evidence also suggestive of separate neural pathways involved in song production and preference learning (Riebel et al. 2002; Bolhuis & Eda-Fujiwara 2003). We suggest therefore that these results highlight the importance of using multiple behavioural (including spatial) metrics when assessing decisions in choice trials (Chapter 2).

Accordingly, the effects of Control vs. Restricted rearing conditions also varied depending on behavioural metric employed. Specifically, zebra finches that were reared in an outdoor environment (Control treatment) revealed a preference for zebra finch songs equally over both the cross-fostered and Bengalese finch songs as measured by spatial discrimination and wing-flick responses. They also showed a preference for zebra finch songs over Bengalese finch songs but not over cross-fostered songs as measured by distance calls. In contrast, the measures of tet calls and song bouts revealed no patterns of behavioural song discrimination, which parallels with previous work on song preference learning by zebra finch males between conspecific and Bengalese finch-like song tutors (Clayton 1988c). In turn, these results are in contrast to male zebra finches showing behavioural preference and song learning from conspecific over heterospecific tutors (Eales 1987b). The Control subjects' conspecific preferences over the phylogenetically close heterospecific songs, as predicted by optimal acceptance threshold theory (Reeve 1989) became more permissive in the Restricted ontogenetic environment, as these subjects appeared to have a reduced pattern of discrimination between similar song types as measured by spatial discrimination, wing-flicks and distance calls.

Although zebra finches reared in social isolation from normal conspecific songs display behavioural preferences for conspecific songs both in adulthood (Lauay et al. 2004) and as juveniles (Braaten & Reynolds 1999) over phylogenetically distant heterospecifics (e.g., Canary: *Serinus canaria* and Starling: *Sturnus vulgaris*), indicating an innate own species bias (Dooling & Searcy 1980), our study suggests that a species-limited rearing environment eliminates behavioural discrimination of phylogenetically related (i.e., estrildid finch: Sorenson et al. 2001) heterospecific songs. This lack of consistent discrimination in our Restricted birds appears to function in parallel to the critical role for a conspecific auditory environment during rearing, including the perception of males' own song, for the development of consistent responses towards conspecific song playback (Pytte & Suthers 1999; Riebel 2000; Riebel 2003). If conspecific auditory stimulation during rearing were needed for discriminatory abilities to develop towards conspecific song then heterospecific stimuli may also be needed during rearing to be able to attend to small differences in related heterospecific song. It must be noted, however, that in our trials the Restricted subjects were

not tested for discriminatory abilities against phylogenetically distant heterospecific songs, just as it is unknown as to whether isolation-reared zebra finches would prefer a conspecific over the songs of estrildid heterospecifics (Lauay et al. 2004). Further research could also address whether exposure to heterospecific vocalisations during adulthood would have shifted the Restricted subjects' response pattern to that of the Control birds, whether multiple or single heterospecific species exposure during ontogenetic development is required for discrimination abilities to develop or whether physical contact with heterospecifics (Eales 1987b) during rearing would have further differentiated discrimination. These are all important possibilities remaining to be explored.

The Restricted environment also critically modulated our subjects' overall engagement in choice-type response behaviours with the subjects showing significantly less response to the presented stimuli, spending a greater proportion of their time in the frozen fear posture (Stripling et al. 2003), producing fewer distance calls and showing distinct behavioural separation in PC1. There were also several Restricted subjects which showed no response at all across all their experimental trials and were therefore excluded from any analyses. This difference in responsiveness, and also in combination with reduced discrimination, could result from greater fear responses to novelty after being raised in a depauperate environment (e.g., Fox & Millam 2004). It is unknown as to whether given more playback repetitions our Restricted subjects would have differentiated between stimuli songs and this warrants further investigation. The difference between a lack of ability to discriminate or a lack of motivation to show behavioural discrimination in our Restricted vs. Control subjects would need to be confirmed by neurophysiological discrimination research (e.g., Hauber et al. 2007a, b). Nonetheless, these behavioural results are still important for sourcing of subjects for future behavioural and neurophysiological discrimination research (Adar et al. 2008), where the rearing conditions can significantly affect overall subject behaviour and therefore may lead to inconsistency in experimental results both within and between research laboratories (Garner 2005).

Our results confirm that zebra finches show discrimination between zebra finch songs and Bengalese finch songs or cross-fostered zebra finches singing Bengalese finch-like songs.

The variation in the captive subjects' response from the different rearing conditions indicates the importance of a greater approximated natural rearing environment which has implications for the sourcing of experimental subjects for future behavioural and neurophysiological song discrimination research.

8

Cross-fostering diminishes song discrimination in zebra finches (Taeniopygia guttata)

8.1 Introduction

Studying the development of avian song production and perception, using the zebra finch (*Taeniopygia guttata*) as a model system (Zann 1996; Williams 2004), has advanced our understanding of the proximate causes of sexual and social behaviours. This small Australasian estrildid songbird has a structured, well-defined set of developmental phases following fledging when the male will learn to produce and prefer songs similar to his tutor's song (typically the genetic father) and the female, which does not sing, will learn to prefer songs of the father's species (typically zebra finch) (Miller 1979b; Riebel 2003b). In the context of acoustic species discrimination, in playback experiments both female and male zebra finches will prefer conspecific songs over those of phylogeographically distant or unrelated species (Clayton & Pröve 1989; Braaten & Reynolds 1999; Lauay et al. 2004). Yet, to date there are few empirical studies regarding an own species bias with respect to song discrimination between conspecific and heterospecific stimuli that include sympatric species (Zann 1996, Chapter 3). Zebra finches in parts of their natural range live in sympatry with and breed in close proximity to several different phylogenetically related estrildid grassfinch heterospecifics (Immelmann 1969). The songs of most grassfinches are relatively similar in structure (Zann 1976) which suggests that the zebra finch would require greater discriminatory abilities to recognise conspecific songs over those of related estrildid songs, than when presented with phylogenetically unrelated heterospecific songs from other oscine lineages (e.g., Braaten & Reynolds 1999; Bailey et al. 2002; Lauay et al. 2004). Greater discriminatory abilities are also predicted to have evolved for species in sympatry so as to maintain reproductive isolation against the risk of costly hybridisation (Coyne & Orr 1989; Price 2008). For a comprehensive understanding of auditory species recognition in zebra finches it is therefore necessary to assess song discrimination using phylogeographically relevant heterospecifics and so using a song playback paradigm, I ask:

1A) *shall zebra finches raised by their own parents recognise and prefer playbacks of unfamiliar conspecific songs over sympatric estrildid heterospecific songs?*

Theory predicts that due to the inequality in reproductive investment between the sexes, females are the more discriminating sex in species recognition (Burley 1977). This was recently confirmed in live-stimulus species discrimination trials on zebra finches that I carried out (Chapter 4). There is also significant sexual dimorphism in both the behavioural development (reviewed in Zann 1996) and neurophysiological control of the zebra finch vocal production and perception system (Wade & Arnold 2004; Hauber et al. 2007b). I might therefore expect variation in the behavioural responses of males and females to auditory song playbacks and in my analyses I also ask:

1B) *shall the behavioural responses to conspecific and heterospecific songs differ between females and males*?

Song preference learning appears to show dual developmental influences: innate predisposition and tutor effects (Hauber & Sherman 2001). Accordingly, in the absence of exposure to adult conspecific song, both female and male zebra finches recognise and demonstrate the presence of innate auditory 'own species biases' for conspecific songs over unrelated heterospecific songs (Braaten & Reynolds 1999; Lauay et al. 2004). However, the direction of preference also critically depends on the acoustic milieu of the early rearing environment where zebra finches in their natural habitat would be exposed to estrildid heterospecific song during song development (Immelmann 1969). Consequently, the fostering of chicks into nests of the domesticated estrildid strain, the Bengalese finch (or society finch: *Lonchura striata* vars. *domestica*), a frequently used laboratory foster species (Sonnemann & Sjölander 1977; ten Cate & Mug 1984; Clayton 1987a, 1988a), results in both male zebra finch production of heterospecific tutor-like songs (Immelmann 1969; Clayton 1988a; Clayton 1989) and female and male spatial and sexual preferences for live Bengalese finches over zebra finches in pairwise choice paradigms (Sonnemann & Sjölander 1977; ten Cate & Mug 1984; Clayton 1987a, 1988a). But similar to the absence of song exposure, Bengalese-fostered zebra finches will also continue to demonstrate a strong response to conspecific song (Immelmann 1969), and a tendency to learn from conspecifics when present in a zebra finch/Bengalese finch mixed flock, implying an own species bias even following fledging from a Bengalese finch nest (Eales 1987a; Clayton 1988a).

However, these previous tests of Bengalese-fostered zebra finches using live stimuli did not assess species song preferences per se as the acoustic cues were not distinct from the visual features. Additionally, no research to date has compared the song-only preferences for own and foster species in comparison to sympatric estrildid songs of both female and male Bengalese-fostered zebra finches. Therefore, I ask:

1C) *shall the species discrimination responses of zebra finches be modulated by social ontogeny*?

In addition to acoustic differences between their songs, zebra and Bengalese finches also display marked differences in parental behaviours, with Bengalese finches showing more parental behaviours overall (e.g., nest-sitting and feeding), less aggression towards young and less singing by fathers (ten Cate 1982). Such disparity of parental behaviours influences the sexual preferences of the offspring (ten Cate 1984; ten Cate et al. 1984), and may have an overall impact on the species discrimination responses of fostered finches which is as yet undocumented in the experimental literature. I use my data to specifically ask:

2) *shall the overall behavioural responsiveness of female and male zebra finches to song playbacks be modulated by social ontogeny*?

For a comprehensive understanding of the strength of conspecific recognition in normal-reared (control) female and male zebra finches and the effect that Bengalese-fostering has on own species bias and discrimination behaviour, I selected song stimuli, in addition to zebra finch and Bengalese finch song, from two phylogeographically relevant estrildid species (Sorenson et al. 2004). These included the closely related owl finch (also known as the bicheno or double-barred finch: *Taeniopygia bichenovii*) and the more distantly related star finch (*Neochmia ruficauda*), as both are sympatric with the zebra finch in its natural habitat in Australia and can be found in mixed feeding flocks (Zann 1996). I employed the methodology of sequential (cf. pairwise) song playback (Zann 1996; Wagner 1998; Hauber et al. 2001) and quantified species discrimination via five different behavioural measures.

8.2 Methods

8.2.1 Study subjects and housing

For my experiments I used 14 adult (Zann 1996) female and 14 adult male zebra finches that had been raised in the University's outdoor aviaries by their own parents and socially exposed to conspecifics only during development (Control). This sample size was deemed statistically informative to detect consistent behavioural preferences, based on my previous work with zebra finch females and males in species discrimination contexts using live stimuli (Chapters 3, 4). The breeding colony of on average 20 pairs was housed in one aviary (3m length x 1.5m width x 3m height) and exposed to the natural photoperiod and local weather conditions in Auckland, New Zealand across approximately 18 months.

For a comparison, a further 14 female and 14 male Bengalese finch fostered zebra finches were reared by transferring chicks (3-5 days old) from their parental nests to the nests of the foster species in my outdoor aviaries (remote from the zebra finch colony so as to be beyond auditory contact). The rearing colony of on average 20 pairs of Bengalese finches was housed in two aviaries (2m x 1m x 2m) and exposed to the natural photoperiod and local weather conditions across approximately 18 months. The foster species were induced to incubate plastic eggs so they were receptive to the newly hatched zebra finch chicks and had no chicks of their own. Thus, fostered birds were reared and song-tutored solely by adult female and male Bengalese finches, with conspecific-only nest mates and no adult conspecific song exposure until experimentation.

All nests of either the Control or Bengalese-fostered subjects contained 1- 6 chicks which is within a range where brood size has been shown to have no effect on song production learning (Gill et al. 2006 but see Holveck et al. 2008, Soma et al. 2006 for tutor group composition effects on song learning). As not all test subjects originated from different broods, there is a possibility of pseudoreplication, but in my analyses I detected no significant effect of brood identity on any of the behavioural measures recorded (detailed below) as assessed by Generalised Linear Mixed Models (GLMM) (all $P \geq 0.16$) (also see

Nelson & Marler 2005 for statistical independence of siblings in other oscine song learning studies). I also acknowledge that the Control subjects were not subjected to a cross-fostering treatment itself (i.e., transferring chicks to broods of conspecific foster parents) as they were raised by their own parents. However, as yet it is unclear from the published literature what precise effects cross-fostering might have on offspring behaviour and perceptual abilities (Forstmeier et al. 2004 but see Boncoraglio & Saino 2008).

All juvenile Bengalese-fostered zebra finches were visually sexed at 35 days (Hauber et al. 2007a) and separated into two Bengalese finch aviaries for further song tutoring, although acoustic contact remained between siblings. One of these Bengalese finch aviaries was the original rearing aviary (either of the two aviaries was designated a rearing aviary for a particular clutch dependent on an incubating pair available for which to give the zebra finch chicks to), and the removal of either male or female siblings for placement into the second aviary was counterbalanced. Upon reaching maturity (~100 days of age, Zann 1996), the Bengalese-fostered zebra finches were removed from their respective tutor aviaries and placed into separate single-sex outdoor aviaries (1.5m x 1m x 1.5m) of Bengalese-fostered conspecifics so all new Bengalese-fostered chicks were exposed to adult Bengalese finch tutors only during their sensitive song-learning phase (Clayton 1987b). All Bengalese-fostered males were judged by human observers to sing Bengalese finch elements (Fig. 8.1: recorded using a Marantz PMD 671 solid state recorder as detailed below); all subjects were sexually mature and naïve to experimentation and came from captive-bred stock due to an exotic bird species import embargo in New Zealand.

All Control and Bengalese-fostered subjects were held in a sound proof chamber on the University of Auckland Campus for the duration of the experimental trials. Control and Bengalese-fostered males and females were brought in from their outdoor aviaries separately. Birds were fed *ad libitum* a commercial finch dry-seed mix (Animates^{TM}) with fresh water provided daily and spray millet, fresh grasses, corn, boiled hen's egg and rice given weekly. Calcium and grit supplements were also provided and all birds were monitored daily with all experiments approved by the University of Auckland Animal Ethics Committee.

8.2.2 Preparation of auditory playback stimuli

Unfamiliar songs from males of 4 zebra finches, 4 Bengalese finches, 2 owl finches and 2 star finches were used for song playbacks (Fig. 8.1). Additional owl and star finches were unavailable due to the rarity of these species in New Zealand. Song recordings of the zebra finches, Bengalese finches, and one star finch were made using a Marantz PMD 671 solid state recorder with a Sennheiser ME66 shotgun microphone, recording the males' song at a sample rate of 44.1 kHz and 16-bit resolution, digitising it directly as a wave file. The owl finch songs were recorded with a Logitech Desktop Microphone (980240-0403) connected to a Toshiba Satellite laptop (A45-S151), running Microsoft Sound Recorder (v. 5.1) with songs stored as wave files (courtesy of C. Hmielewski). The second star finch recording was obtained from R. Beckham (www.efinch.com) with recording details unknown.

Playback stimuli were edited in Raven Sound Analysis Software v.1.2.1 (Charif et al. 2004). All songs were bandpass filtered 500-16000 Hz except for the owl finch songs which were received filtered at 8000 Hz (for equality all spectrograms display filtering at 8000 Hz, Fig. 8.1) and amplitudes were root mean square equalised. All introductory notes to songs were removed and four motifs of song (within each stimulus species) (Holveck & Riebel 2007) were repeated at approximately 10 s intervals for the 10-min playbacks (see below).

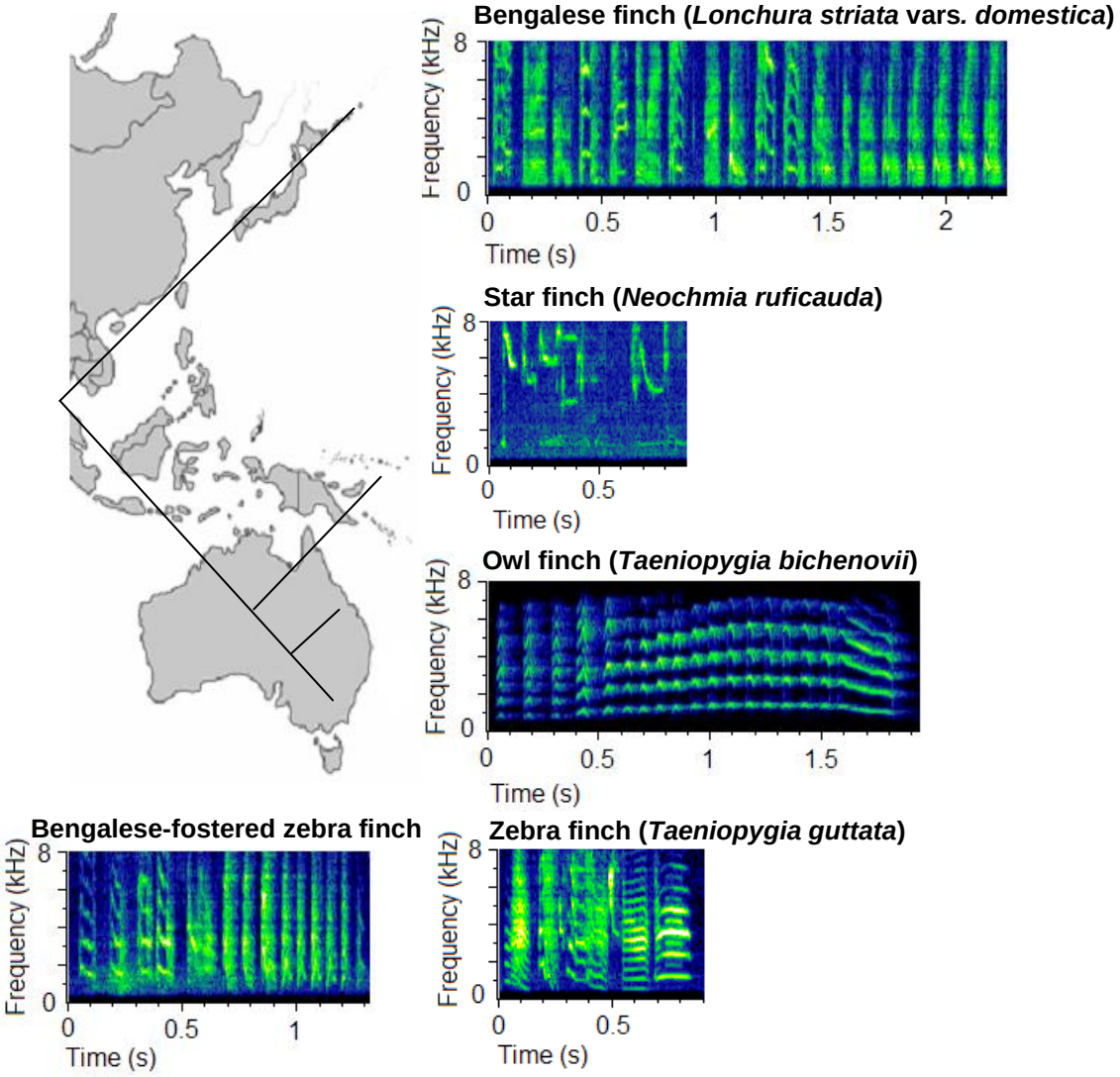


Figure 8.1: The phylogeographic distribution and frequency versus time spectrograms of single song motifs of the estrildid stimuli used in my experiments, including a song motif from a Bengalese-fostered male subject.

8.2.3 Experimental set-up

Sequential (cf. pairwise) song playback trials were conducted on the university campus from February-April 2008. The room was fluorescently lit with an ambient temperature of 20 ± 2 ^{o}C. The testing apparatus consisted of a single cage (length 2.0 m, width 0.6 m and height 1.67 m) in which a polystyrene cage base was placed horizontal inside the cage 0.87 m from the ground to reduce cage height to 0.8 m (see Fig. 7.2). The cage was divided with 5 perches labeled P1-5 (length 0.23 m, placed at height 1.12 m) of which 2 were placed 0.53 m from the cage ends and the other 3 evenly distributed in between. P1 and P2 or P4 and P5 (and the immediate surrounding ground area; P1 and P5: 0.65 m, P2 and P4: 0.235 m) were classed as side-proximity or discrimination zones depending on which side the speakers were placed. P3 was classed as a central neutral zone. A table clothed in white fabric (1.70 m x 0.76 m x 0.93 m) was placed 0.05 m from the test cage. Two small Radioshack™ AMX 3 speakers were placed on a box directly in front of (distance of 0.30 m) and level with P1 or P5 (in random) also clothed in white fabric. A larger box (for size equality) was placed in front of the opposing end perch and also clothed in white fabric so the subjects had no visual cues for associating with the auditory stimulus (see Fig. 7.2). A Dell latitude D620 laptop was used for all auditory playbacks and was located behind the observers' screen. Auditory playbacks as measured at the centre of P1 and P5 using a digital sound level meter (Radioshack™ 33-2055), were delivered at an amplitude/volume that fell between the normal range of male zebra finch song amplitudes at this distance (60-68dB SPL), as measured in the University's aviaries. Volume equality was maintained for all heterospecific song playbacks. The volume was re-measured each day before trials commenced to confirm that the correct sound level was maintained.

Food and water *ad libitum* were placed in the centre of the cage, opposite P3 and the entire set-up was located behind a fabric screen positioned 1.0 m from the test cage with mesh holes to permit the observer to watch and record all trials unseen (see Fig. 7.2).

8.2.4 Experimental procedure

All trials (duration 10 min.) were run daily from 12 midday local time onwards to adhere to the same time schedule across trials with all subjects. I allowed 24 hr for each individual subject to become familiarised with the test cage prior to experimentation. The subjects were removed after their acclimatisation period and then individually placed in the test cage before trials, allowing 15 min. to adjust. This schedule enabled several subjects to be tested during a single day as only 4 consecutive playback trials were run at a time with each subject to minimise the possible effects of lack of social/sexual motivation on behavioural responses.

Discrimination by female and male Control and Bengalese-fostered zebra finches to sequential song playbacks was quantified through multiple behavioural responses. These consisted of (1) spatial association, which is a commonly employed discrimination metric (e.g., Forstmeier & Birkhead 2004), (2) soft, short 'tet' calls which are frequently produced and suggested to be close contact calls between individual zebra finches (Zann 1996), (3) louder, longer 'distance' calls which are produced in several contexts including excitement or sudden danger (Zann 1996), for males only (4) song bouts which are produced in response to song playbacks (Stripling et al. 2003) and are frequent in wild flocking contexts (Dunn & Zann 1996), and (for both sexes) also (5) response latency (seconds) which was the delay from the onset of the playbacks until subject movement of a $\geq$ 30 s duration (i.e., preceding these movements, the subjects were typically frozen in a state of tonic immobility presumably induced by a response to novel stimuli: Gallup 1979; Bischof & Lassek 1985; Stripling et al. 2003).

During each trial, the perch location and behaviour (Zann 1996) of the subject was recorded using a scan-sampling design every 10 s (one record of location and behaviour per scan). All behaviours were recorded by the same observer (DLMC). In addition, the total number of tet and distance calls and song bouts produced during each trial were noted. The subject was left without a playback for a 15 min. break then the second trial was run. This procedure was repeated for 4 trials for the particular subject and they were then replaced

with a new subject who was left for 15 min. before their first trial was run. Each subject was observed over sequential presentations of 4 trials per stimulus species songs using two different song files for each species presented once on each test cage side. A total of 16 trials were run for each subject (896 trials in total). Individual songs, species and test cage side presentation were randomised and counterbalanced.

8.2.5 Statistical analyses

The data were summarised to provide (1) the proportion of total time that individual subjects spent in spatial proximity to the auditory stimulus (either P1 and P2 combined or P4 and P5 combined), the total number of (2) tet and (3) distance calls and (4) song bouts (males only) that they produced (in any area of the test cage), and their (5) total response latency time across trials with the same stimulus types. The data for each behavioural measure were averaged across all trials for each subject in response to each of the four stimulus species' songs.

All proportional data were initially log transformed ($\log_{10}(X + 1)$), and used in Generalised Linear Mixed Models (GLMM) ($\alpha < 0.05$) in JMP 7 (Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA). A *post hoc* Student's t-test was used on the least squares means estimates of the response variables for each behavioural measure. All proportional and count data were standardised (X-mean/SE) for principal component analysis (PCA, see below).

For figures I present the raw values of all behavioural measures and PC1/PC2 in response to each stimulus species for Control and Bengalese-fostered subjects (Statview 5.0.1: Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA).

8.3 Results

8.3.1 Discrimination of song playbacks by zebra finches: effect of subject sex, stimulus species, and ontogeny on behavioural measures of discrimination

Female and males differences (irrespective of ontogeny) in response to the stimulus species songs were assessed by exploring the effect of sex on the calculated difference between the transformed proportions of spatial discrimination data, the numbers of tet calls, distance calls and the response latency for all stimulus species songs using GLMM with subject identity added as a random effect. Contrary to predictions, there was no significant effect of sex on any of the behavioural response measures towards each stimulus species across all subjects (spatial discrimination [$F_{3,81}$ = 1.37, P = 0.26], tet calls [$F_{3,81}$ = 1.06, P = 0.37], distance calls [$F_{3,81}$ = 0.06, P = 0.98], response latency [$F_{3,81}$ = 2.25, P = 0.09]). Therefore, data for female and male subjects were pooled for all subsequent analyses.

As predicted, Control subjects significantly preferred to spatially associate with the conspecific (zebra finch) songs (Fig. 8.2a). The experimental treatment of ontogeny had a significant effect on the spatial discrimination responses towards each species ($F_{6,162}$ = 2.96, P = 0.0092) (Fig. 8.2a), with Bengalese-fostered subjects, contrary to expectations, spatially associating with zebra finch, owl finch and Bengalese finch songs equally and more so compared to the star finch songs (Fig. 8.2a).

Control subjects did not produce significantly more tet calls in response to the zebra finch songs over the other species songs (Fig. 8.2b). There was no significant effect of ontogeny on the number of tet calls produced in response to each stimulus species ($F_{6,162}$ = 1.30, P = 0.26) (Fig. 8.2b).

Control subjects produced significantly more distance calls in response to the zebra finch songs over the owl finch songs but not over the star finch and Bengalese finch songs. There

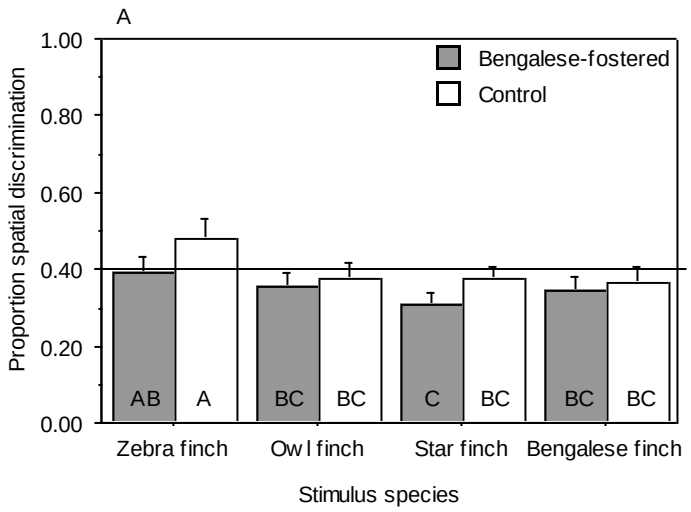
was a significant effect of ontogeny ($F_{6,162}$ = 2.19, P = 0.05), with Bengalese-fostered subjects producing significantly more distance calls in response to the zebra finch songs over the owl finch and Bengalese finch songs but not to the star finch songs (Fig. 8.2c).

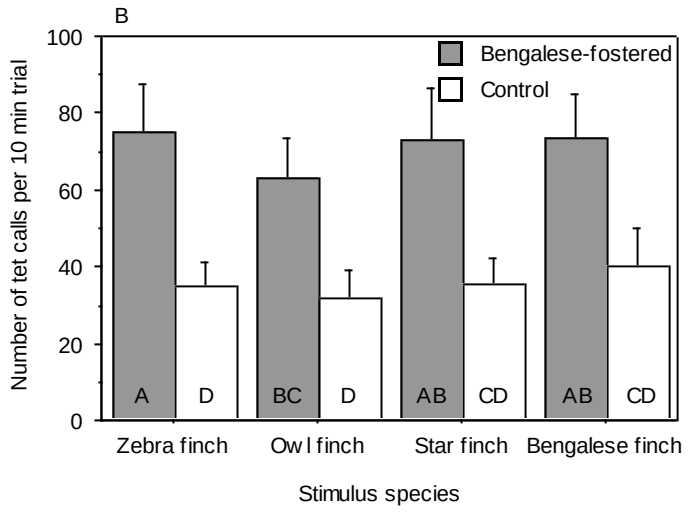
Male Control subjects produced significantly more song bouts in response to both the zebra finch and star finch songs over the owl finch and Bengalese finch songs (Fig. 8.2d). This response was significantly affected by ontogeny ($F_{6,78}$ = 2.75, P = 0.018), with Bengalese-fostered subjects producing equal numbers of song bouts between all stimulus species (Fig. 8.2d).

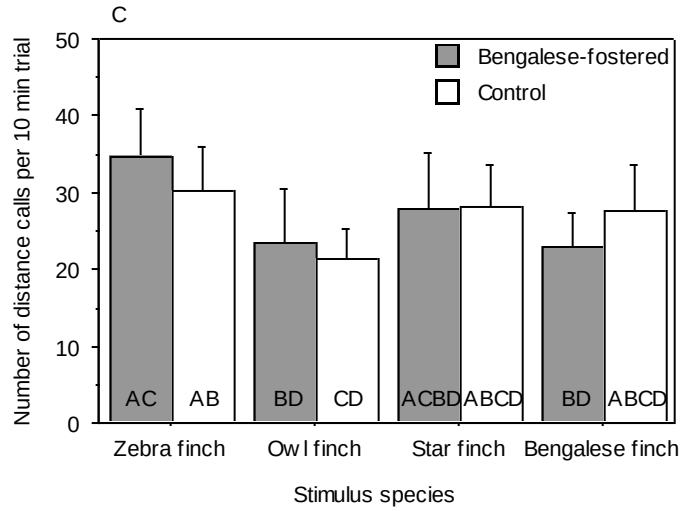
Finally, Control subjects responded more quickly to all species songs over the owl finch songs (Fig. 8.2e). There was no significant effect of ontogeny on this response latency variable ($F_{6,159.9}$ = 1.85, P = 0.092) (Fig. 8.2e).

8.3.2 Effect of early social environment on behavioural responsiveness to song stimuli

Contrary to predictions, ontogeny did not significantly affect the subjects' overall spatial discrimination responses towards all stimulus species songs combined ($F_{1,54}$ = 1.12, P = 0.30) (Fig. 8.2a). Bengalese-fostered subjects did, however, produce significantly more tet calls than the Control subjects ($F_{1,54}$ = 7.31, P = 0.0091) (Fig. 8.2b), but did not differ from Control subjects in the number of distance calls produced ($F_{1,54}$ = 0.0023, P = 0.96) (Fig. 8.2c). Control subjects sang more ($F_{1,26}$ = 4.63, P = 0.041) (Fig. 8.2d) and Bengalese-fostered subjects responded to all stimuli significantly more quickly ($F_{1,53.69}$ = 5.66, P = 0.02) (Fig. 8.2e).


A
Bengalese-fostered
Control
Proportion spatial discrimination
1.00
0.80
0.60
0.40
0.20
0.00
AB
A
BC
BC
C
BC
BC
BC
Zebra finch
Owl finch
Star finch
Bengalese finch
Stimulus species


B
Bengalese-fostered
Control
Number of tet calls per 10 min trial
100
80
60
40
20
0
A
D
BC
D
AB
CD
AB
CD
Zebra finch
Owl finch
Star finch
Bengalese finch
Stimulus species


C
Bengalese-fostered
Control
Number of distance calls per 10 min trial
50
40
30
20
10
0
AC
AB
BD
CD
ACBD
ABCD
BD
ABCD
Zebra finch
Owl finch
Star finch
Bengalese finch
Stimulus species

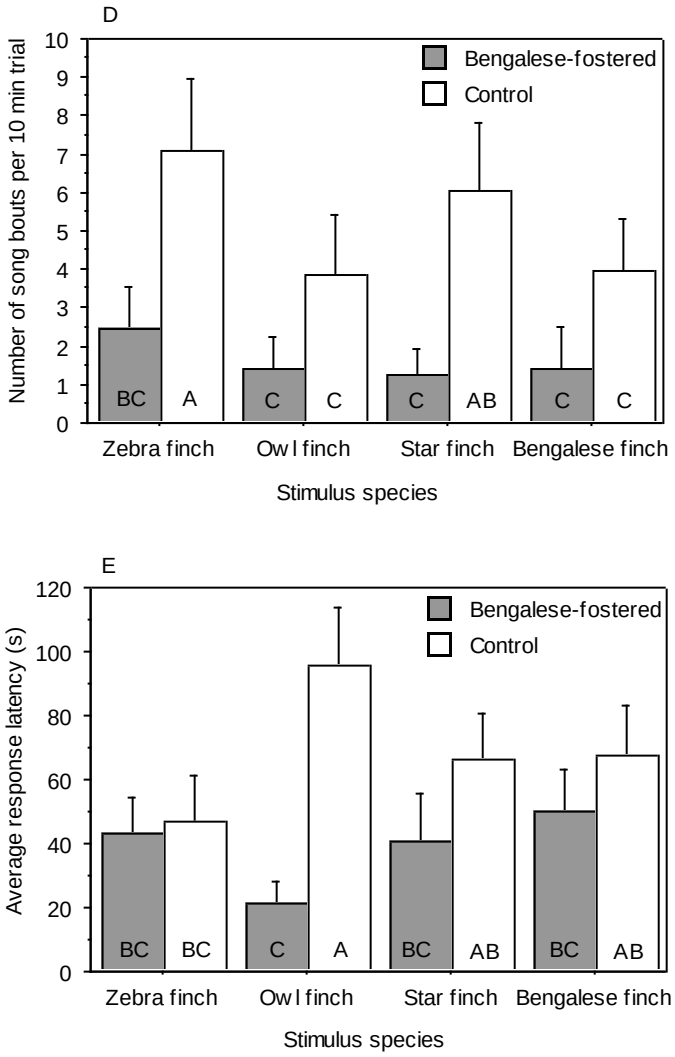


Figure 8.2a-e: The average proportions or numbers for Bengalese-fostered (n = 28) and Control (n = 28) subjects for each response variable in the presence of each stimulus species' song (a: spatial discrimination, b: tet calls, c: distance calls, d: song bouts (males only), e: response latency). Mean of the raw values + SE depicted where solid line (a) indicates random choice of 40% (each perch had a random expectation of 20%). Species in combination with ontogenetically dissimilar subjects that do not have the same letter showed significant *post hoc* differences.

8.3.3 Principal component analysis

A principal component analysis was used to reduce the variation across all behavioural response variables, excluding the variable of number of song bouts for which data was available for the male subjects only. The first principal component (PC1) explained 40% of the variation in the data (eigenvalue 1.59), and the second principal component explained 26% of the variation in the data (eigenvalue 1.03) accounting for 66% of the total variation. Response latency was negatively associated with PC1 (Eigenvector: -0.59) and all other response variables were positively associated (Eigenvectors: spatial discrimination: 0.48, tet calls: 0.49, distance calls: 0.41). Tet calls were negatively associated with PC2 (-0.63) whereas all other variables were positively associated (spatial discrimination: 0.26, distance calls: 0.71, response latency: 0.18).

Species discrimination: effect of sex, stimulus species and ontogeny: There was no significant effect of sex on PC1 ($F_{3,80}$ = 0.96, P = 0.41) or PC2 ($F_{3,80}$ = 1.05, P = 0.37) towards each stimulus species for Control and Bengalese-fostered subjects. The GLMM analysis revealed that Control subjects, as shown by PC1, displayed significant discrimination against the owl finch and star finch songs over zebra finch songs but not against the Bengalese finch songs (Fig. 8.3a). Ontogeny had a significant effect on the species discrimination responses (PC1: $F_{6,162}$ = 2.94, P = 0.0095), with Bengalese-fostered subjects in contrast showing no discrimination between all species songs (Fig. 8.3a). Control subjects, as shown by PC2, displayed no discrimination between stimulus species' songs, and ontogeny had no significant effect ($F_{6,162}$ = 1.47, P = 0.19) (Fig. 8.3b).

Behavioural responsiveness and early social environment: There was no significant effect of ontogeny on overall behavioural responsiveness as shown by PC1 ($F_{1,54}$ = 2.65, P = 0.12) (Fig. 8.3a). There was a significant effect, however, on PC2 ($F_{1,54}$ = 5.00, P = 0.030) with Control subjects showing positive association and Bengalese-fostered subjects negative association (Fig. 8.3b).

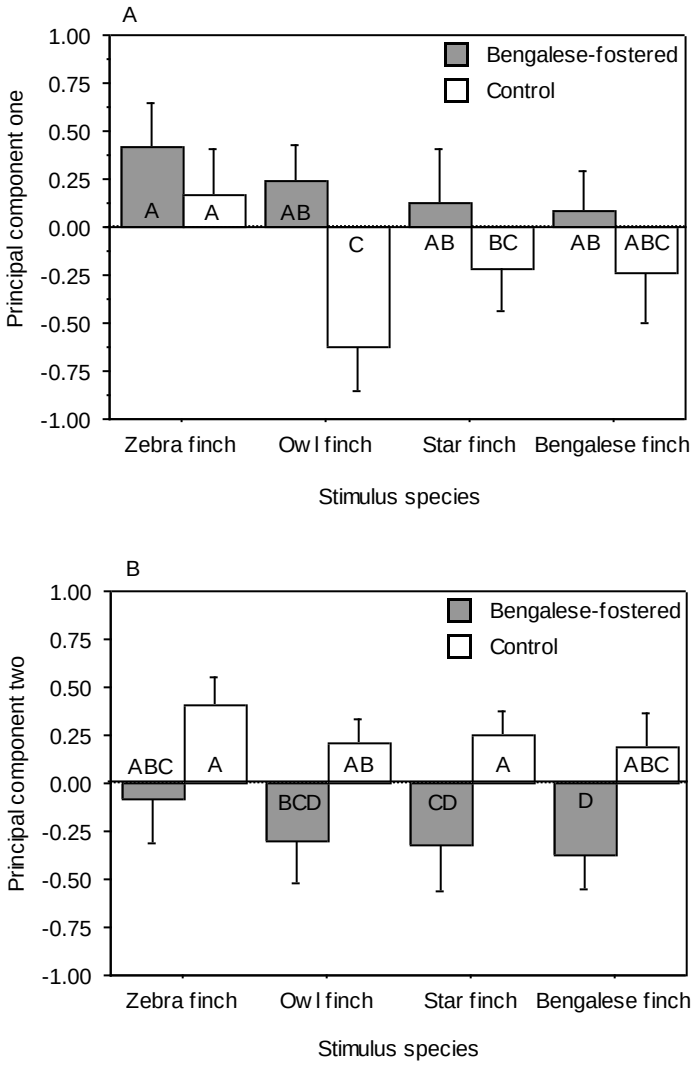


Figure 8.3a, b: The responses of Bengalese-fostered (n = 28) and Control (n = 28) subjects in the presence of each stimulus species' song in relation to principal component one (a) and principal component two (b). Mean of the principal component values + SE depicted. Species in combination with ontogenetically dissimilar subjects that do not have the same letter showed significant *post hoc* differences.

8.4 Discussion

The results of my behavioural song discrimination trials with Control and Bengalese-fostered zebra finches in response to con- and heterospecific song playbacks of related estrildid finches indicate that there is equality between the sexes in discriminatory abilities and there is an effect of rearing environment in reducing song discrimination in adulthood. However, there is also great variability in the behavioural responses of zebra finch subjects to con- and heterospecific song playbacks depending on the response measure employed.

The lack of significant differences between male and female subjects was surprising given the vast differences in the neurophysiological and behavioural song systems of the sexes (Zann 1996; Wade & Arnold 2004). Females with the smaller song nuclei might be less proficient in song recognition (Wade and Arnold 2004) and male production of song can alter perception (Pytte & Suthers 1999). In contrast, regarding the published data on behavioural tests comparing perceptual song discrimination abilities in both sexes (reviewed in Riebel 2003a), my finding conforms to previous studies that have also failed to demonstrate sex differences in both song and syllable discrimination (Braaten & Reynolds 1999; Riebel et al. 2002; Braaten et al. 2006 but see Vicario et al. 2001) in playback trials. Both females and males learn to prefer specific songs during the sensitive period of development (Clayton 1988b) and likely longer where females learn to recognise the songs of their mates (Miller 1979a) and males use song for potential individual recognition (Clayton 1988b) and male-male communication (Riebel et al. 2002). Perceptual abilities are therefore important for both sexes and this behavioural data on heterospecific song preferences along with the fact that the Bengalese-fostered males produced a Bengalese finch song but did not prefer it provides possible behavioural evidence for the separation of neural pathways involved in song preference and song production learning in this species (Riebel et al. 2002; Gobes & Bolhuis 2007; Hauber et al. 2007b).

The multiple behavioural measures that were employed for quantifying song preferences in this experiment produced varying results and indicate the importance of using several different metrics (Rutstein et al. 2007). Such variability is expected given the different

ecological context in which each behaviour would be produced in the zebra finches' natural social environment (Zann 1996) and although this may result in findings that are less definite than studies using single metrics (e.g., spatial association: Forstmeier & Birkhead 2004), it provides a more comprehensive assessment. The variation indicates that the song discrimination is a complex cognitive process and I cannot conclude which behaviour is most suitable or accurate for assessing preferences but instead suggest that multiple measures enhance overall understanding.

For the purposes of interpretation I focus here on the result of the principal component analysis which showed that Control zebra finches, based on PC1, preferred the songs of their own species over the sympatric songs presented but not the domesticated, more distantly related Bengalese finch songs. This matches my phylogeographic predictions that these sympatric relatives of zebra finches (Sorenson et al. 2004), which breed in close proximity (Immelmann 1969; Zann 1996), are discriminated against perhaps within the context of reproductive isolation to be maintained (Coyne & Orr 1989; Price 2008). Additionally, this result has important implications for stimulus selection for future behavioural and neuroethological studies with zebra finches as a model species, because the responses obtainable in a species discriminatory paradigm may depend on the choice of stimuli and their evolutionary relevance to the subject taxon (Hauber & Sherman 2001; Göth & Hauber 2004; Theunissen et al. 2004). PC1 also revealed that the manipulation of the rearing environment had a significant effect on the species responses with Bengalese-fostering reducing the discrimination leading to no display of preferences. PC2 in contrast revealed no preference in Control subjects and no effect of the rearing environment.

The reduced discrimination in the Bengalese-fostered subjects was surprising and contrary to what I predicted where previous studies that used Bengalese-fostering demonstrated sexual preferences for the foster species (Sonnemann & Sjölander 1977; ten Cate & Mug 1984; Clayton 1987a, 1988a), and so I initially predicted that my Bengalese-fostered subjects would, in turn, show a consistent preference for the Bengalese finch song. However, I did not test behavioural contexts of sexual preferences *per se* (i.e., copulation solicitation displays: Clayton 1990b) or use live stimuli for courtship, mating, or pair

bonding (Rutstein et al. 2007). Therefore, the discrepancy between my song discrimination results and previous live stimulus preferences suggests that both visual and vocal cues modulate species recognition and mate selection in zebra finches (Brazas & Shimizu 2002) and that the sensory processes of sexual imprinting and song development are distinct recognition systems (Clayton 1988a; but see ten Cate et al. 1993). The lack of clear species-specific song discrimination using several behavioural metrics in my Bengalese-fostered subjects suggests that although zebra finches display an innate bias for conspecific song (Immelmann 1969; Braaten & Reynolds 1999; Lauay et al. 2004; Hauber et al. 2007a), the consolidation of this initial own-species bias into a fixed preference requires exposure to adult conspecific song (Pytte & Suthers 1999). The exposure of the Bengalese-fostered subjects to a tutor song different to their innate predisposition may have lead to subject confusion, similar to previous behavioural discrimination trials with mismatched cues (Galoch & Bischof 2006; Chapters 5, 6), resulting in the lack of song discrimination. This is important for zebra finches in their natural habitat where upon fledging they are exposed to the songs of related heterospecifics and need to maintain contact with adult conspecific males for accurate song discrimination learning to occur.

In addition to an effect on discrimination, the manipulation of social ontogeny had an effect on overall behavioural responsiveness of the subjects to the presented song stimuli which is consistent with the transference of differing parental behavioural traits to offspring (ten Cate 1984; ten Cate et al. 1984). For instance, the overall lower song rate of the Bengalese-fostered subjects suggests they copied the lower song production rate of Bengalese finch adult males during song development (ten Cate 1982).

In conclusion, my experiment on song discrimination in zebra finches and the effects of early social environment revealed that zebra finches can discriminate conspecific song in comparison to closely related stimuli but that fostering by a heterospecific reduces the extent of behavioural responses of discrimination and the use of multiple behavioural measures is recommended for future research on song development and song perception learning in this model species.

9

General conclusions

9.1 Implications of experimental data, limitations and future research

The question of how individuals are able to accurately recognise members of their own species is fundamental to the understanding of the evolution of sexual reproduction (Göth & Hauber 2004). Biologists from diverse specialties strive to decipher the mechanisms of the recognition of conspecific cues over a multitude of heterospecific cues present in the environment. In this dissertation I contributed to knowledge on the model species of the zebra finch (*Taeniopygia guttata*). I used phylogeographically close and distant stimuli to document how the strength of recognition varies depending on the biological relevance to the subject, whether visual and acoustic features are important and in which way is the ontogenetic environment critical for behavioural discrimination. My research contributes new insights yet there are still many questions that warrant further investigation as will be addressed here.

9.2.1 Chapter 2

The introductory review on laboratory methods of ecological research (Chapter 2), discussed the many values of a captive setting over field work for greater control of experimental context, subjects and stimuli. Such control enables, for example, the elucidation of specific features of stimuli that are involved in correct species identification, or the manipulation of subject ontogeny to assess the effects of learning and varying juvenile social contexts on adult behaviours. But it is precisely this artificiality of the captive environment that limits the interpretation of findings and their applicability to subjects in their natural habitat. These limitations might include, for example, eliminating visual, acoustic or physical interactions with additional conspecifics (i.e., audience effect, Vignal et al. 2004) during choice trials that would otherwise be influential to decisions made in the wild. Therefore, many of the findings from my experiments in the latter chapters would benefit from either further work carried out in the field or parallel experiments with wild-caught birds, a component of zebra finch research that is currently underrepresented (Rutstein et al. 2007). One possibility might be to broadcast song playbacks to wild colonies of zebra finches. This would allow a comparative analysis of

responses to songs of estrildid heterospecifics that they may encounter on a day-to-day basis in contrast to, for example, songs of the domesticated Bengalese finch or of estrildids from African lineages, which would be biologically irrelevant to them in the context of hybridisation-avoidance. These playbacks would also permit the observation of interactions between wild zebra finches in response to heterospecific songs to document if approach or retreat behaviours are dependent on the responses of other conspecifics or if there is any difference in the response behaviour either between males and females or between adults and juveniles.

Executing research in the laboratory setting simplifies the stimulus/response relationship but can also exclude these and other socioecologically important variables. More conclusive research findings can always stem from a combined field and laboratory approach.

9.2.2 Chapters 3 and 4

The experimental data of Chapter 3 resulted from simultaneous conspecific versus heterospecific live stimulus choice trials. These were done using female zebra finches as subjects and zebra finch males matched with a range of phylogeographically close or distant estrildid heterospecific males, including the typically used Bengalese finch, as stimuli. The data showed that female zebra finches, irrespective of the heterospecific taxa presented, preferentially spatially associated with and behaviourally displayed more towards conspecific males. The proportions of behavioural displays towards the heterospecifics did, however, vary depending on taxa presented. The parallel experiment of Chapter 4 used male zebra finch subjects and female zebra finches matched with females of the same heterospecific taxa as stimuli. These data showed that male zebra finches did not preferentially spatially associate with either conspecific or heterospecific females but did behaviourally display more towards the conspecific female stimuli. The proportion of time spent displaying towards the conspecifics or heterospecifics also varied depending on the heterospecific stimuli presented. In both sets of trials, response to novel estrildids didn't consistently differ from the response towards the Bengalese finches validating the use of this heterospecific stimulus in prior and future work.

The zebra finches used (Chapters 3 and 4) had been exposed during evolution to estrildid heterospecifics but there is a possibility that domestication of zebra finches, the likely reduced genetic variation of the strains available for this research (due to the avian importation embargo in New Zealand), and the captive rearing of zebra finches with social exposure to conspecifics only, influenced the observed behaviours (cf. wild-caught subjects). Potentially, in the absence of such confounds the strength of social preference would increase towards exclusivity for conspecifics. Future work based on the findings from this research might involve comparisons of the behavioural preferences of subjects who have had previous experience with estrildid heterospecifics during development to determine if this influences adults' discrimination behaviour. This could be through either exposure in their natural environment (wild-caught adult zebra finches) or through controlled exposure to selected estrildid heterospecifics in a captive environment (Dukas 2008). Continuing research on captive finches could also determine if the timing of exposure to heterospecifics, either during development or adulthood, is important. Furthermore, research could establish if the exposure needs to include physical interactions with the heterospecifics (e.g., residing in the same aviary) such as is required for juvenile zebra finches during song learning (Eales 1987a), or if visual and acoustic exposure would be sufficient (e.g., residing in neighbouring aviaries) for increased species discrimination accuracy.

The findings of Chapters 3 and 4 are also critical for research laboratories in which the majority of zebra finch subjects are sourced from captive, domesticated stock. Captive zebra finches showed behavioural preferences for live conspecifics but caution should be applied when selecting stimulus species in future research as the heterospecifics' phylogeographic history with the zebra finch may lead to variation in the strength of behavioural species discrimination and its neural basis (Hauber et al. 2007a, b).

The potential of mating status (i.e., being pair bonded or not) of subject and stimuli individuals having influence on social preference was a recognised confound in both Chapters 3 and 4. The comparison between mated and unmated Bengalese finch stimuli

(Chapter 3) showed no significant effect of mating status on spatial association of female subjects. But there were no behavioural measures taken between these two types of Bengalese finch stimuli (Chapter 3) and only mated female stimuli were presented to the male subjects (Chapter 4), while all subjects were unmated in tests of discrimination (both Chapters 3 and 4). These mating status variables all warrant further attention in social choice trials.

Zebra finches form strong pair bonds and there is relatively little evidence as to what extent this alters their behaviour towards opposite sex conspecifics in the absence of their mated partner. A question of interest would be whether mated stimulus birds behave similarly to unmated stimulus birds. Factors relevant may be: motivation to interact with the subjects, or general activity levels where it might be predicted these factors would decrease in already mated stimuli. If there were such differences in behaviour depending on mating status, it would remain to be determined how long individuals need to be separated from their mate before behaving as an unmated individual would (Silcox & Evan 1982). Furthermore, and perhaps most importantly, would such variation in stimulus behaviour influence the discriminatory decisions made by the subjects? Similar (to Chapters 3 and 4) behavioural observations during pairwise choice experiments using conspecific stimuli only, mated versus unmated, would provide answers to these questions. Alternatively, subjects could be presented with four stimuli individuals, a choice between a mated pair and an unmated pair of conspecifics. This would help to determine if any behavioural differences in the interactions between the stimuli (e.g., varying levels of pair bonded behaviours or early courtship behaviours) would influence discrimination by the subject and if that subject would preferentially respond towards the unmated pair that might potentially provide a mating partner.

A further experimental set-up that would provide new insights in such tests would involve sound attenuation and one-way glass to exclude subject/stimulus interactions. This set-up would isolate subject response to stimuli and eliminate the potentially confounding variable of stimulus response to the subject. Such a protocol would also provide confirmation of whether variations in behaviour are important for mate preference decisions and to what

degree subject/stimuli interactions influence the choices made. Alternatively, video playbacks can be conducted (see Chapter 5 below).

In addition to the effects of stimuli mated status on discrimination, the variation in the behavioural displays of both female and male subjects, depending on mating status is a research avenue to be explored. It would be predicted that motivational factors for mate selection might be diminished in already mated subjects. Sexual selection theory would predict variation between females and males, where males might be as equally motivated to associate with a mate as to associate with a partner for extra-pair copulations. Finally, it could be explored whether the strength of the pair bond depends on breeding experience (Delesalle 1986). Would stimuli or subjects be more likely to behave as being mated, after separation from their partner, if they have successfully raised at least one clutch together? This is a factor that would particularly highlight the importance of knowing the breeding history of all individuals used for experimentation.

All these possible questions would hold relevance for further behavioural choice experiments and increase our understanding of the factors that may modulate social preference behaviour in the model species, the zebra finch.

9.2.3 Chapters 5 and 6

The experiments of Chapters 5 and 6 involved further dissection of the cues that are available to and used by female zebra finches when making recognition decisions. This research investigated the relative roles that visual and/or acoustic stimuli play during spatial selection. In Chapter 5 I presented video playbacks of zebra finch males or sympatric plumhead finch males, separating playbacks of acoustic cues and visual cues and mismatching these cues between the two stimulus species. In Chapter 6, live stimulus males of either white or wild-type colour morphs producing either zebra finch or Bengalese finch-like vocalisations were presented to female subjects. The data from these two experiments showed that both visual and acoustic features were important in the recognition and selection process and that these needed to be accurately matched with species identity for a

female to make accurate conspecific preference decisions. Mismatching of species-specific features resulted in no spatial preference for either stimulus-type presented. This potentially contrasts with other zebra finch research that indicated the acoustic features were sufficient for making accurate discrimination decisions (Holveck & Riebel 2007), as was also confirmed by my data in Chapter 8. Generally there is very little known about the precise role of visual cues for accurate discrimination and zebra finch recognition research would benefit from further investigation in this area.

Video playbacks can provide a useful tool for manipulating visual stimuli and specifically, the research questions from Chapter 5 could be expanded by using video playbacks of several more estrildid stimulus species. In particular, there needs to be more data on female preferences using visual-only playbacks. An extended training period (greater than three hours) with video playbacks might demonstrate more conclusively whether females are able to discern silent video images and if these captivate to the same extent as acoustic playbacks. Additionally, there should be particular focus on presenting an increased variety of different heterospecific finch visuals that are manipulated to sing zebra finch songs. This might lead to more conclusive and general findings on both female recognition of video images and the relative importance of visual vs. acoustic cues for species recognition. However, as video screens are not designed for avian vision, an alternative method might be through the use of moving finch models or robots (Patricelli et al. 2002). These could be designed in a similar manner to present in isolation, the visual cues from different estrildid species, and to mismatch visual and acoustic features. Furthermore, these models could be used for investigating bimodal cue spatial integration (Narins et al. 2005). Broadcasting acoustic and moving visual model cues at varying spatial disparities (Narins et al. 2005) could determine at what point bimodal integration occurs and zebra finch subjects regard the visual and acoustic cues as one stimulus. This would provide data on the perceptual limits of zebra finches for future tests on scale and role of distance in the natural habitat. The colonial breeding and mixed-species feeding flocks of zebra finches mean daily encounters with many individuals and/or species within short distances and the sensory precision that is needed for the accurate recognition of conspecifics or mates.

Overall there is great scope for multimodal (Partan & Marler 2005) species recognition research with zebra finches using video playbacks or moving models as presentational tools.

Although there is less capacity for manipulation of visual and acoustic cues using live stimuli, there are multiple colour morphs of male zebra finches that might be useful in experimental work determining the roles of this plumage colouration in social and mate selection (Hauber et al. 2000). These morphs include variation in cheek patches (e.g., black-checked zebra finch) and chest bands (e.g., orange-breasted zebra finch) which are sexually attractive features for females. In addition to future mate choice tests with different colour morphs, the findings of Chapter 6 could be greatly expanded on by implementing free-flight pair bonding or breeding success studies (Swaddle 1996). These experimental methods may provide more conclusive evidence of sexual preferences per se and confirm the relative roles of colour manipulations and heterospecific-like vocalisations on female social choice. In general, many zebra finch choice test paradigms could benefit from further free-flight pair bonding experiments to confirm the sexual motivation of subjects' preferences in the initial tests.

The individuality of behavioural responses as shown by female subjects during video playback discrimination (Chapter 5) and the subsequent correlation with subjects' likelihood to form a pair bond with test males, indicated complexity of the mate selection process and the importance of individual behavioural variation. The consistency of behaviours from one context (video playback discrimination) into another (pair bonding) provides possible evidence for personalities or stable traits, an aspect of zebra finch research that is stimulating increased interest (e.g., Boogert et al. 2008). This is crucial for the understanding of more features and cues that cause individuals to prefer one conspecific over another and future research should aim to determine the individual consistency and developmental origins of these behavioural traits. Are personalities stable across multiple testing scenarios, varying on a scale of boldness versus shyness? For instance, bolder individuals might be more aggressive towards other conspecifics, more responsive to novel stimuli, or show overall greater activity levels during testing. Additionally, are these differences based on genetic variation, parental effects or environmental effects? Would

cross-fostered genetic siblings or unrelated nestmates show similar behavioural traits? Furthermore, would behavioural traits depend on the number of siblings or density of conspecific exposure during development (Adar et al. 2008)? If personalities were stable across multiple testing scenarios, then to what extent would this influence sample selection? One might predict that bolder individuals may be more likely to show a response in the testing paradigm over shyer individuals and this could potentially bias behavioural choices or population-based conclusions. The possibility of stable personalities is important for discrimination research and the degree to which variation in behavioural traits might alter attractiveness of stimuli over the physical features (e.g., song or plumage) that are being assessed. Furthermore, if zebra finches select on the basis of matching personality, would a bolder female be more likely to pair with a bolder male? Overall, there is great scope for exploration of behavioural variation and its influence on social discrimination at all levels of analyses.

9.2.4 Chapters 7 and 8

The final data Chapters 7 and 8 limited the number of sensory modalities available to subjects. They explored female and male behavioural responses to song playbacks (only) of conspecifics and heterospecifics and the effect that the developmental environment had on these preferences. In Chapter 7 I conducted a test of the optimal acceptance threshold hypothesis using sequential song playbacks. I compared the behavioural responses of zebra finches raised in outdoor aviaries with exposure to conspecific and heterospecific songs (Control), to the behavioural responses of zebra finches raised in indoor aviaries with exposure to conspecific songs only (Restricted). The playback stimuli included song of zebra finches, Bengalese finches and Bengalese finch-fostered zebra finches. Multiple behaviours were measured which showed that being reared indoors, with exposure to the songs of conspecifics only, reduced the subjects' ability to discriminate between the songs of conspecifics and heterospecifics. No consistent variation was found between female and male responses (irrespective of ontogeny). Control subjects showed preferences for conspecific songs but did not discriminate between the Bengalese-fostered zebra finch and Bengalese finch songs, while Restricted subjects showed no preferences between any of the

three song types. This supports the optimal acceptance threshold theory in that a lack of comparative stimuli during development for the Restricted subjects increased their acceptance rate for heterospecific stimuli.

In Chapter 8 I employed a cross-fostering manipulation to assess the role of the sensory environment of early development on behavioural choices in adulthood. I compared the responses of zebra finches to sequential song playbacks for subjects either raised by their own parents (Control) or raised by heterospecific Bengalese finch foster parents (Bengalese-fostered). Song stimuli were selected from two biologically relevant sympatric finch species, the owl finch and the star finch (in addition to parental and foster species' songs), to assess the effects of phylogeography on species discrimination. Similar to the findings of Chapter 7, being reared by a heterospecific reduced the displays of discrimination between the songs of conspecifics and heterospecifics, and there was little variation between the responses of females and males (irrespective of ontogeny). Control subjects preferred the songs of conspecifics but there was variation in response to the other species' songs across the different behavioural measures. Bengalese-fostered subjects did not prefer the songs of their foster species as was predicted by previous studies of sexual imprinting and song learning in Bengalese-fostered zebra finches (e.g., ten Cate & Maug 1984; Clayton 1988a, 1989).

A critical finding across both these experiments (Chapter 7 and 8 and also Chapters 3 and 4) was that the song discrimination results varied depending on the behavioural measures employed. The multiple behaviours recorded for both experiments did not produce consistent patterns of discrimination. Nonetheless, the value of this technique is that it comprehensively assesses the complexity of the discrimination process and potentially avoids missing important behavioural information. Future behavioural research should employ the recording of multiple behavioural traits and possibly increase the range of behaviours observed. Although spatial discrimination is already a widely accepted metric, a meta-analysis of future literature could identify other behavioural measures that consistently reveal social preferences.

In addition to varying results depending on the behavioural measure used, Chapter 7 showed that discrimination against heterospecifics was dependent on the stimulus taxa presented. Similar to the experiments with live stimuli (Chapters 3 and 4), song stimuli of species that are biologically relevant to the zebra finch were used and which had been previously untested in zebra finch auditory discrimination research. There has been much focus on determining whether zebra finches of different ages and manipulations can distinguish between songs of conspecifics and songs that are acoustically considerably different (e.g., European starling: *Sturnus vulgaris* or canary: *Serinus canaria*; Braaten & Reynolds, 1999; Lauay et al., 2004). But to test the evolutionary strength and limits of acoustic discrimination it is necessary to present songs that are both acoustically similar and would be produced by phenotypically similar birds (i.e., other estrildids). By using these estrildid stimuli I have demonstrated that a preference for conspecific song exists but that not all heterospecific songs are discriminated against equally. Further studies of species recognition in zebra finches should use multiple heterospecific stimuli and in particular, an increased range of estrildid species. Specifically exploring to a greater extent how behavioural discrimination might vary between Australian and other taxa.

A sequential song playback paradigm was used for both these experiments leaving the potential for replication using pairwise song presentations to assess preferences. This would be particularly valuable for documenting relative discrimination strength (Wagner 1998) between different heterospecifics when a conspecific option is eliminated. Yet, both sequential and simultaneous presentations measure preferences via passive behavioural discrimination. Further research with ontogenetically manipulated subjects could employ supplementary testing in the alternative paradigm of operant training (Riebel 2000). Subjects would be trained to the auditory reward of their preferred species' songs. The operant technique allows subject control over song presentations and requires active participation (Riebel 2000). This might, in particular, reveal whether the two treatment groups, Restricted and Bengalese-fostered (cf. control groups) did indeed have preferences, but were going to require longer exposure/training with the song stimuli to be able to make their discriminatory decision. Consistency in both passive and active measures would enable stronger conclusions to be drawn.

These final two experiments (Chapters 7 and 8) showed that the rearing environment of the subjects significantly affected later discriminatory preferences. This was particularly interesting in Chapter 7 as rearing zebra finches in a single-species controlled indoor colony is not unusual for zebra finch research. This raises significant concerns about the validity of multiple past (and ongoing or future) experiments using subjects that respond differently to zebra finches raised with acoustic exposure to heterospecifics (Chapter 7). Research should focus on additional effects (other than song discrimination) of social exposure to a single species during ontogeny to document the extent of influence of this unnatural rearing environment. Further avenues for exploration might involve whether song production differs between males raised with exposure to single or multiple species (but when still learning from a conspecific tutor only). Alternatively it could be studied whether these subjects are any less adept at making species discriminatory decisions when presented with live stimuli as compared to zebra finches raised with heterospecific social exposure.

To expand on the sensory limits of development, fostering zebra finches to other estrildid species which are phylogeographically more relevant than Bengalese finches, or rearing with conspecific or robotic tutors producing heterospecific songs, would provide more information on the limits of song preference and production learning and the extent to which the early environment affects later preferences. Heterospecific foster parents or tutors should comprise a range of phylogeographically diverse species to assess if their geographic relationship to the zebra finch affects their learning. Acoustic analysis of tutor and learned songs could determine copying accuracy and if zebra finch temporal structure is always retained as per Bengalese finch song copying.

These ontogenetic effects on discrimination behaviours stress the importance of knowing the developmental history of all subjects and stimuli that are used for zebra finch behavioural research (Spencer & Verhulst 2007).

Finally the results of the acoustic playback trials not only hold relevance for further behavioural research with zebra finches but also provide useful information for zebra finch

neuroethological research (Theunissen et al. 2004). This is a research field based heavily on acoustic playbacks and how zebra finches perceive their own species' songs over those of heterospecifics, including the specific features of songs (e.g., temporal order of notes) that are critical for discrimination. The importance of stimulus selection on behavioural responses should be considered for neural responses where there is currently a lack of data using a range of heterospecific estrildid songs. My research showed a lack of discrimination in ontogenetically manipulated subjects but I cannot conclude if this was caused by a lack of perceived differences in the song stimuli or a lack of behavioural motivation to display choice. Neuroethological studies would advance these conclusions and determine what features of heterospecific songs induce physiological responses distinct from those towards conspecifics songs (e.g., note structure or temporal structure).

9.3 Conclusion

The experiments that comprise my dissertation have provided results that contribute to the overall understanding of species recognition and mate selection in the model system of zebra finches. Sympatric estrildids are relevant to the zebra finch and species identity does have an effect on discrimination. Therefore, the more frequent use of these related estrildids as stimulus species is recommended. Both visual and acoustic cues are important for species discrimination but there is great scope for further investigation on specifically the role of visual cues. An increased use of multiple behavioural measures when assessing preferences is also recommended to ensure a more comprehensive interpretation of the discriminations observed. Ontogeny is critical to zebra finch social choices where both the parental species and the surrounding acoustic environment during development have significant effects on subsequent species preferences. These findings are critical for future experimentation with zebra finches and potentially other species as without knowing the full ontogeny of subjects and stimuli, the results obtained can only be interpreted with limited generality.

Future behavioural experiments with zebra finches should also involve tests with wild-caught subjects, use phylogeographically relevant stimulus species and explore more extensively the effects of early social environments.

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