On the production and perception of syntactical regularities in zebra finches : experimenting with ABBA, ACDC and others

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On the production and perception of syntactical regularities in zebra finches: Experimenting with ABBA, ACDC and others

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Contents

Chapter 1

Introduction

The language faculty

One of the major differences between humans and other animal species is language. ‘Language is a cognitive system, which uniquely allows humans to produce and comprehend meaningful utterances (regardless of the mode of communication of those utterances)’ (Tserdanalis and Wong 2004). Moreover, human language enables us to create a limitless number of sentences from only a limited number of elements. To find out how such a complex phenomenon could have evolved has fascinated scientists as well as the general public for a long time. For many years it was thought that *all* features characterizing language were present in humans only. But in more recent years, more and more mechanisms underlying language production and perception that were once thought to be unique to human have also been found in animals, such as categorical perception (in chinchilla’s (*Chinchilla laniger*) (Kuhl and Miller 1975) and vocal learning (in songbirds, hummingbirds, parrots, bats and cetaceans) (Janik and Slater 1997; Doupe and Kuhl 1999).

The search for the uniquely human aspect of language got a new direction after Hauser, Chomsky and Fitch published a controversial paper in 2002. They made a distinction between a broad and inclusive faculty of language called FLB, (the faculty of language in a broad sense, including the sensory-motor system, conceptual-intentional and possible other systems) and a more restricted and narrow faculty of language that is part of the FLB, but specifically adapted for language, called FLN (Faculty of Language in the Narrow sense). One of their hypotheses was that a core property of this narrow sense language faculty is ‘recursion’, an aspect of abstract syntax that enables us to make a potentially infinite number of different expressions with only a limited number of elements. Later, in 2004, Fitch and Hauser broadened this hypothesis towards the use of Phrase Structure Grammars as the only aspect of language that is unique to humans. Phrase Structure Grammars are more complex than Finite State Grammars because they can produce infinitely long, hierarchical sentences, including recursion and nonadjacent dependencies like ‘if…then’, while Finite State Grammars cannot. These grammar types are part of the ‘Chomsky hierarchy’ that orders these and other different grammar types according to their complexity. (See for more information on the Chomksy hierarchy: Chomsky 1957). As other scientists disagreed for various reasons on whether this narrow sense aspect of language would contain recursion, or later, Context Free Grammars only, this new approach in comparative research inspired a new and ongoing scientific debate and flow of research articles (see for instance Fitch *et al*. 2005; Pinker and Jackendoff 2005; Bolhuis *et al*. 2010). It also raised the question whether the use of more complex syntax structures is indeed limited to humans only, and if so, how this evolved.

The question of language evolution is one of the most difficult questions in science: language does not fossilize and therefore analysis of our pre-hominid ancestors cannot reveal what the key feature was that makes human language so complex compared to animal communication. Hauser *et al*. (2002a) therefore proposed to use the comparative approach using empirical data from living species to draw inferences about extinct ancestral species, like Darwin already proposed (Darwin 1859), but now specifically in the context of language evolution.

From this evolutionary viewpoint, the logical starting point for most scientists would be to study the communication systems of non-human primates, as they are our closest relatives. Non-human primates have an elaborate system of non-verbal communication. However, they do not have the complex learned vocal signals that we have, neither do they have the expressive and open-ended power of human language (Hauser *et al*. 2002a). But when we turn to other species in the animal kingdom, it has been found that at least a number of other mammals (cetaceans and bats,) and birds (parrots, humming birds and various songbirds,) do have complex, learned vocal signals (Janik and Slater 1997; Jarvis 2004). So even though the last common ancestor between humans and birds was an amniote and therefore very distant (pre-reptilian even, see Meyer and Zardoya 2003; Jarvis *et al* 2005), studying these more distant related species can provide us with insights into common mechanisms and possible precursors that have resulted in the complex cognitive mechanisms involved in language.

Birdsong development and parallels with human language development

From a functional perspective, bird song generally serves territorial defense and mate attraction (Bradbury and Veherencamp 1998; see for a review on birdsong Marler and Slabbekoorn 2004). In general, high quality song signals a high quality male. High quality males are better at defending their territory and in producing offspring. Song is therefore a very important fitness characteristic for songbirds. Many songbird species produce songs that can be decomposed into several units. In case of the zebra finch, a widely used model species in neuro-and behavioural biology and also the subject of this thesis, these units are called notes or elements, which together form a motif. Motifs in turn are grouped into song bouts. A male's song can contain multiple different element types in a relatively stereotyped element order, or phonological syntax, and the element type repertoire can differ between individuals. (Immelman 1969; Sossinka and Böhner 1980; Cynx 1990;

Scharff and Nottebohm 1991; Zann 1993a, b and 1996; Catchpole and Slater 1995; Lachlan *et al.* 2010).

Many parallels exist between human language development and songbird song development, such as a sensitive phase for vocal learning early in life; imitation of adults, a babbling phase in which utterances are practiced, a need for auditory feedback, a predisposition to be tuned to species-own sounds and specific hierarchical neural substrates for vocal learning (Doupe and Kuhl 1999; Jarvis 2004). These similarities make birdsong the closest animal analogue to human language, or at least the best experimentally accessible one. So additionally to the possibility to answer questions about the origin of language from an evolutionary viewpoint (the comparative perspective), these parallels between the human and songbird system enables us to answer questions from a language developmental perspective, especially where human experiments cannot be done for ethical reasons. Songbird experiments can therefore provide information on for instance developmental and neurological mechanisms involved in vocal learning and vocal production (Doupe and Kuhl 1999).

Production of syntactical rules

In human language, syntax considers the ordering of meaningless phonemes (part-words) into meaningful words and in turn into meaningful sentences. A different order of these units can therefore result in a different meaning, even though the units from which the sentence is built are identical. There is no substantial evidence to date that animals change the meaning of their utterances by changing the order comparable to human language flexibility (Berwick *et al*. 2011, but see Arnold and Zuberbühler 2006).

Syntax in the context of bird song research can be defined in general as restrictions in the sequencing or position of structural units (Lachlan *et al*. 2010). What is important to mention, is that there is no reference to meaning in the context of bird song syntax and that over the years descriptions of bird song syntax have used terms such as ‘song complexity’, ‘song variability’, ‘song stereotypy or -linearity’, either focusing on constraints in sequence order or the opposite, variation in sequence order, or the repertoire size of an individual bird. The degree of variability in the sequence of song elements differs greatly between bird species, with the reed warbler (*Acrocephalus arundinaceus*) as an example of a relatively high element sequence variability (Catchpole 1983) and the nightingale (*Luscinia megarhynchos*) as a widely known example of high song sequence variability (Todt and Hultsch 1996). Various methods have been employed to describe and analyse the patterns in variability in an individual’s song, such as transition diagrams for Bengalese finch song (*Lonchura striata* var. *domestica*) (Honda and Okanoya 1999), first and second order Markov models in chick-a-dee-calls (*Parus atricapillus*) (Hailman and Ficken 1986) and even higher level Markov modeling for rose-breasted grosbeak song (*Pheucticus ludovicianus)* (Lemon and Chatfield 1973).

This thesis is about the natural variation in zebra finch song structure, or phonological syntax, as well as the ability of zebra finches to detect and distinguish between different types of syntactic structures in artificial stimuli consisting of zebra finch song elements. In doing so, it addresses questions left open in other comparative studies in humans and other animals on the perception of syntactical rules. This study on production and perception of syntax in zebra finches will therefore be of interest to both the fields of zebra finch research and comparative artificial language learning.

Zebra finch song syntax

For zebra finches, earlier analyses of song syntax have focused on two different types: the intra-individual (or song repertoire) level and the inter-individual (or population) level in which the song characteristics are compared between birds.

Intra-individual (song motif repertoire) level: Initially Immelmann (1969) stated that each male sings in an invariant order, but over the years this has shifted to the general opinion that adult male zebra finches sing relatively stereotyped but with slight differences in the order of elements (Sossinka and Böhner 1980; Scharff and Nottebohm 1991; Volman and Khanna 1995; Sturdy *et al.* 1999a; Helekar *et al.* 2000). See fig 1.1 for an example of a zebra finch song. A set of quantitative measures was developed by Scharff and Nottebohm (1991): ‘sequence linearity’ (ordering of elements, independent of frequency of occurrence) and ‘sequence consistency’ (relative occurrence of a particular order). But a more intuitive measure was used by Sturdy *et al.* (1999a) and Helekar *et al.* (2000) called the ‘predominant motif’: the note order with the highest probability for that

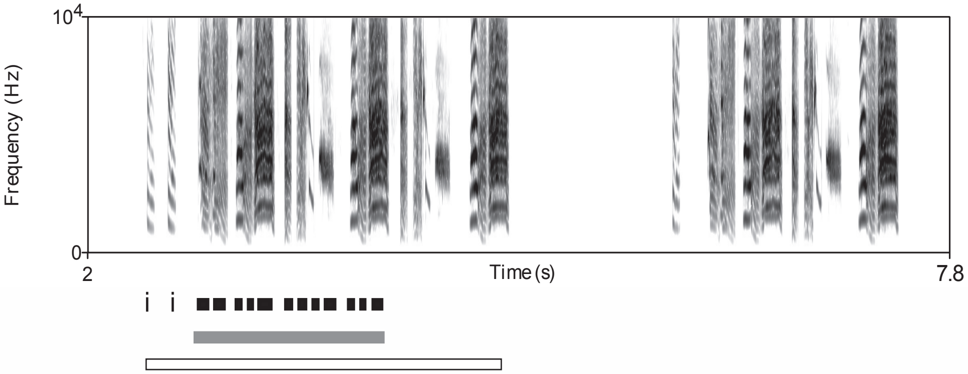

Fig 1.1. Example of zebra finch song. The i’s represent introductory elements. Black bars indicate elements. The grey bar indicates a motif. The white bar indicates a song bout.

bird. Songs deviated from the predominant motif by skipped, added, altered or repeated elements, mostly in the end part of the motif.

Inter-individual (population) level: Even though there is relatively little intra-individual variation in syntax, there is more variation in where element types can occur in the song of different individuals. Zann (1993a, b and 1996) did an 'absolute syntax' analysis and divided the song in three temporal parts. He suggested that introductory elements occurred more frequently at the start, high and compound elements in the middle and distance call notes and stacks/ flats at the end part. Contradicting this finding, a more extensive study on three captive populations did not find such a classification for relative position in the song (Lachlan *et al*. 2010). In relation to phonological syntax, Lachlan *et al*. (2010) analyzed transition probabilities between neighbouring elements and found that they were not randomly distributed in the data of four populations.

Inter-population level: As zebra finches learn their song from other males in the group, transferring over multiple generations, this is a form of cultural transmission. Therefore, apart from genetic differentiation between zebra finch populations (Forstmeier *et al.* 2007), the process of cultural evolution can also give rise to differences in zebra finch song between different (lab) populations, which might be called dialects (Petrinovich and Baptista 1984; Catchpole and Slater 1995). This can be the case for both element types and syntax. Some element types or syntactical rules might occur in all populations, while others only occur in a limited number of populations due to isolated reproduction and learning in the various lab populations. According to Zann (1993b) differentiation between the wild populations is not as large as could be expected, but this might be different for domesticated zebra finches.

Since the 1960's, domesticated zebra finches have been an increasingly popular model species for neuro- and behavioural research. Instead of repeatedly acquiring wild individuals directly from Australia to maintain a stock, research laboratories and commercial breeders around the world have successfully bred many generations of domesticated zebra finches. Until now, only a relatively limited number of zebra finch populations from various labs have been analyzed for element types (four populations, by Slater and Clayton 1991) and syntax regularities (three, by Lachlan *et al*. 2010) However, it is still unknown how element types and syntax vary overall between the relatively large number of lab populations of zebra finches in the world and whether rather separated populations on different continents differ from each other in these song characteristics (North America, Australia and Europe).

Therefore, one aim of this thesis was to carry out a large scale study on the natural variation in elements and syntax in zebra finch song. Such a study will be of great value to zebra finch researchers. It will provide a valuable background for all sorts of studies on the (neuro)biology of song and song learning in this widely used model species and it will provide an assessment of comparability between lab populations and wild zebra finches. Moreover, it provides new and comparative information on cultural diversification between populations and hence be of relevance to the fields of (human) cultural evolution and dialect formation.

Perception and rule learning in zebra finches

Production and perception might run in parallel, but that need not be the case. In a number of species it has been found that they are able to perceive specific syntactical rules in acoustic stimuli even though their natural vocalizations do not follow such regularities. Examples of this are found in primates like chimpanzees (*Pan troglodytes*) (Endress *et al*. 2010), rhesus monkeys (*Macaca mulatta*) (Hauser and Glynn 2009), cotton-top tamarins (*Saguinus oedipus*) (Hauser *et al*. 2001; Newport *et al*. 2004), but also more distantly related species such as rats (*Rattus norvegicus*) (Toro and Trobalon 2005; Murphy *et al*. 2008), starlings (*Sturnus vulgaris*) (Gentner *et al*. 2006) and Bengalese finches (*Lunchura striata* var. *domestica*) (Abe and Watanabe 2011, but see Beckers *et al.* 2012).

A number of animal studies have been inspired by work in infants and language development addressing the question whether the mechanisms involved in the detection of rules or regularities in speech are more general cognitive mechanisms or are specific for language (Saffran *et al*. 1996; Marcus *et al.* 1999). How humans detect regularities in sequences of (speech) sounds is extensively studied in the field of Artificial Grammar Learning (AGL). Topics in this field are how adults detect regularities in sequences of speech sounds (or stimuli in other modalities) and how this rule learning ability develops in children. Hauser *et al*. (2002b) did a comparative AGL study on tamarins inspired by the infant studies mentioned above that tested the ability to detect the abstract structure rule that was underlying a series of three-syllable strings. However, this article was retracted recently, after an internal examination at Harvard University 'found that the data do not support the reported findings' (Hauser *et al*. 2010). A number of years later, Murphy *et al*. (2008) tested whether rats could detect similar rules in artificial stimuli. Additionally, two comparative papers on artificial rule learning inspired by the paper by Hauser *et al*. (2002a) tested whether tamarins and starlings could discriminate between sequences following a Finite State Grammar and sequences following a Phrase Structure Grammar (Fitch and Hauser 2004; Gentner *et al*. 2006 respectively).

However, these studies have been criticized from various perspectives (e.g. Marcus 2006; Corballis 2007a and 2009; ten Cate and Okanoya, in press). The concerns involve the nature and modality of the stimuli (conspecific versus heterospecific); the extent to which the stimuli resemble actual language structures; the issue of generalization to new stimuli and the extent to which there is sufficient verification of which rule exactly has been learned by the animal(s). Consequently, the question to what extent animals are able to detect syntactic or structural rules is still open.

Therefore, in addition to describing the natural variation in zebra finch song syntax, it was our aim to examine whether zebra finches can perceive regularities in strings of vocal elements arranged according to patterns also employed in studies on artificial language learning, specifically addressing the questions raised in the debate on artificial language learning in animals.

In this thesis I explored both the structure of their own songs (**Chapter 2**) and the perception (**Chapters 3 and 4**) of syntactic rules by zebra finches from a comparative perspective. Zebra finches are an excellent model species as they are a widely used model species in behavioural biology, neurobiology and more recently in biolinguistics to study parallel processes in vocal learning and development in humans and songbirds. In **Chapter 5** I give an overview of the results and discuss the main findings (synthesis).

Chapter 2

Zebra finch song phonology and phonological syntax across populations and continents

C.A.A. van Heijningen, S. M. ter Haar, R.F. Lachlan and C. ten Cate

C.A.A. van Heijningen and S.M. ter Haar share first authorship.

ABSTRACT

The zebra finch (*Taeniopygia guttata*) is a widely used model-species. Many neuro- and behavioural biology laboratories maintain a local population of these song birds. In this paper we present an extensive study of the phonology and phonological syntax from 13 different populations from Europe, North America and Australia. Our aim was to explore whether, in addition to the well-established individual variation in songs, there are more or less species-wide 'universals' as well as systematic variation among populations. By using a computational approach, we found 11 element types occurring among all 13 populations. The only commonality in terms of sequences of elements seems to be the position of long flat elements towards the end of the song. Despite these commonalities there is considerable variation between populations in the distribution of element type frequencies. For syntax, 8 out of 13 populations had a significant amount of structural constraints in their songs. However, these constraints were absent on a species-wide and continent level. These findings may be important for interpretation of results from different populations and other (bird) species used in experimental research. Moreover, they are suggestive of cultural differentiation being at least as important as genetic diversification processes in causing large scale changes in song characteristics.

Manuscript

Chapter 2

INTRODUCTION

Birdsong and language exhibit many parallels. First of all both communication systems rely on learning. Human infants as well as young songbirds must be exposed to adult vocalizations in order to properly learn the sounds. Second, predispositions for species specific aspects of the sound seem to play a role in the learning process. Third, both song and language learning go through a babbling stage in which auditory feedback shapes vocal development. And as a last parallel, songbirds and humans posses specialized brain mechanisms for vocal production and learning. Together, this evidence suggests that there may be similar mechanisms underlying the learning of birdsong and language (Doupe and Kuhl, 1999; Bolhuis *et al*. 2010).

Another similarity, and relevant to this paper, is that both songs and language consist of rapid sequences of differently sounding vocal elements, organized according to specific patterns. In this paper we present an extensive study of the phonology and phonological syntax (restrictions in sequences of elements, independent of differences in meaning) of a songbird species, the zebra finch. Our aim is to explore whether, similar to language, underneath the well-established individual variation in songs there are more or less species-wide 'universals' as well as systematic variation among populations.

Studying both universal and non-universal song features may tell us more about the relative roles of experience dependent and independent factors on vocal development and on the way sound patterns are organized Birds of different populations can have different 'dialects' if birds from the same population have a more similar song (or lement repertoire) than birds from neighbouring populations often related to geographic variation. Differences between populations have been found in for instance, white-crowned sparrows (Marler and Tamura 1964; Petrinovich and Baptista 1984) and black-capped chickadees (*Poecile atricapillus*) (Kroodsma *et al*. 1999) showing distinct sound patterns between neighbouring populations, related to the geographical distance and social interaction between individuals. Over generations such populations may diverge even further, changing their vocalizations because of reduced exchange between populations combined with cultural transmission with slight modifications within populations. Cultural transmission processes may, however, also help to conserve sound patterns, and this, combined with biological factors such as perceptual biases and production constraints, may limit the types of patterns that are possible, leading to some song features that may occur in many (but not necessarily all) populations ('universals') whereas others occur in a few.

Zebra finches are an excellent model system for studying how song patterns may start to differentiate between populations, for which parameters, and which mechanisms cause these changes. Since the 1960's, domesticated zebra finches (*Taeniopygia guttata*) have been an increasingly popular model species for neurobiological and behavioural research. Instead of repeatedly acquiring wild individuals directly from Australia to maintain a stock, research laboratories and commercial breeders around the world have successfully bred many generations of domesticated zebra finches. This process thus creates an unprecedented large scale experiment that allows us to examine both the universal song features present across al populations as well as how strongly song features have diverged among populations. Here, we take a first step towards addressing this issue by providing a detailed description of the variation among 13 populations.

The process of domestication had consequences for the genetic and cultural evolution of these (laboratory) 'populations'. The genetic relatedness between 18 of these laboratory populations from North America and Europe and two wild populations from Australia was analyzed by Forstmeier *et al*. (2007). They found that populations differed significantly according to broad geographic relationships: the most pronounced genetic differences occurred between the three continents. In addition, domesticated birds were found to differ phenotypically from wild zebra finches, being physically larger than wild zebra finches. In conjunction with this genetic differentiation over the years, cultural evolution may have led to differentiation in learned song characteristics. Repeated (and at least partly isolated) breeding at research laboratories might have lead to the formation of dialects comparable to dialects in isolated populations in other bird species. Comparing the genetic analyses with the variation in songs among different populations and continents can provide insight in whether songs in genetically more similar populations are also more similar in song features or not.

Furthermore, it is yet unknown whether the song of domesticated zebra finches still reflects the natural variation of wild populations or whether domestication has lead to differences in song characteristics compared to those of wild birds. For instance, it is known for Bengalese finches (*Lonchura striata* var. *domestica*) that the process of domestication of white-backed Munia's (*Lonchura striata)* originating from China into Bengalese finches by Japanese breeders (starting approximately 260 years ago) resulted in more variable intra-individual element transitions and hence much more variable songs (Honda and Okanoya 1999).

In the current study we examine the variation and universal patterns of phonology and phonological syntax in zebra finch song from wild and laboratory populations and whether the differentiation among populations has parallels with the genetic differences found between continents.

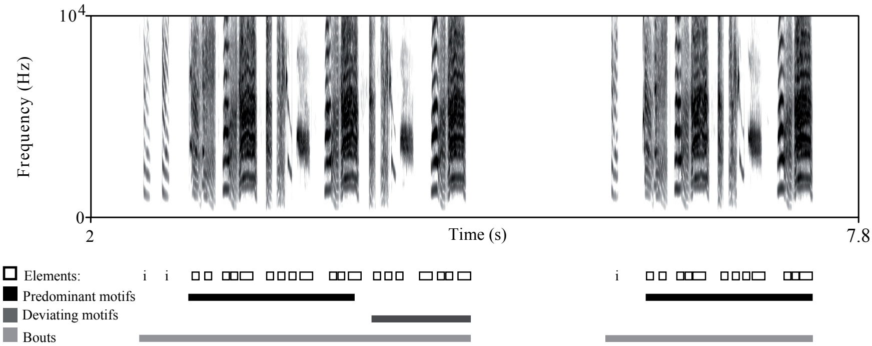

Fig 2.1. Example of a zebra finch song. A song consists of bouts (light grey) of which the predominant motif (black) is selected for further analyses. The predominant motif is the version occurring most often in an individual's repertoire, compared to a less often occurring one like the deviating motif presented here (medium grey). Introductory elements (i) were not taken into account in this study.

Phonology

Many studies on song variability have focused on 'phonology'. By phonology we mean the study of the smallest units of which a song consists: 'elements' or 'notes', which are usually categorized by visual inspection of spectrograms (fig. 2.1. See the Method section for an overview of the different categorization schemes in the zebra finch song literature). In our study, we follow the usual convention of studies on song bird phonology (e.g. Sturdy *et al.* 1999a and b; Prather *et al.* 2009; Lachlan *et al.* in prep. See also ten Cate *et al.* in press) by focusing on the signal itself instead of the phonological/categorical perception that has been demonstrated for some species (Nelson and Marler 1989). Phonological variation between local populations in the type of elements has been shown in several species, for instance in white crowned sparrows (Nelson 1998). For the zebra finch, some comparative element type studies have been done (Slater and Clayton 1991; Zann 1993a, b and 1996; Sturdy *et al.* 1999a; Lachlan *et al.* 2010). These studies were limited in scope, however.

In the present study we examine the presence and distribution of element types on a much wider scale and rather than relying on visual inspection and categorization, we use an advanced computerized element categorization algorithm.

Phonological syntax

Syntax in the context of bird research can be defined in general as restrictions in the sequencing or position of structural units (e.g Lachlan *et al.* 2010). The degree of variability in the sequence of song elements differs greatly between bird species. For example, Chipping sparrows (*Spizella passerina*) have a single song, consisting of one repeated element Albrecht and Oring (1995) while on the other side of the complexity spectrum, the nightingale is a wide known for it's relatively high individual sequence variability (Todt and Hultsch 1998). Various methods have been employed to describe and analyze the patterns in variability, such as transition diagrams (Honda and Okanoya 1999) and (higher order) Markov Models (Hailman and Ficken 1986; Lemon and Chatfield 1973; see also ten Cate and Okanoya, in press, for an overview). But most such methods have been concerned with investigating variability within individual repertoires, rather than variation between different populations.

In zebra finches, individual males were previously thought to sing in bouts of largely invariant motifs (Glaze and Troyer 2006; Immelmann 1969; Zann 1996). But the current general opinion is that although males sing relatively stereotyped songs, they can vary on this pattern by adding, deleting or sometimes altering elements in the motif (Sossinka and Böhner 1980; Scharff and Nottebohm 1991; Volman and Khanna 1995; Sturdy *et al*. 1999a; Helekar *et al*. 2000). Additionally, Helekar *et al*. (2000 and 2003) found relatively many repeated elements in songs from their lab population, a phenomenon that has been observed in isolate song (raised without a tutor) as well (Jones *et al*. 1996; Helekar *et al*. 2000).

On the inter-individual (or population) level, there is far more variability, more specifically in where element types can occur in the song. Lachlan *et al*. (2010) found high variability in the syntactic patterns as all element types can be followed by each other. This is in contrast to an earlier study by Zann (1993 a, b and 1996), who found patterns for element types relative to the position in the song (start, middle or end). He suggested that song elements are not equally distributed over the song: the first section consists mostly of introductory elements, the middle section consists of a 'high' element and 'stacks' and 'distance calls' occur more towards the end of the song (Zann 1996). Thus, previous studies have shown diverging outcomes with respect to the variation and common patterns that can be found in zebra finch song.

In the present study we provide an extensive species-wide analysis including both phonology and phonological syntax on data from 12 laboratory populations and one wild population across three continents. Apart from an analysis at population level, we also did one on continent level to examine whether Forstmeier *et al*.'s (2007) finding of greater genetic similarity within than between continents also holds for song similarity. Computational bio-acoustic analyses are performed on zebra finch song to answer the following questions for both phonology and syntax:

1) What are the species-wide element types and are they present in all populations (i.e. are they universal) or do populations differ in this?
2) What is the degree of species-wide phonological syntax and how consistent is this across populations (i.e. are they universal) or do populations differ in this?
3) Are there differences between continents and how do these relate to population differences?

Chapter 2

METHODS

Song collection

Songs were obtained by contacting various research groups with laboratory populations of zebra finches, many of which generously send us recordings. We aimed for samples of songs from 15 to 20 individuals per location, from normally raised males (without song learning manipulations) and preferably without father-son relations. Songs were recorded by using a variety of recorders and microphones, and sound quality varied. Not all songs provided were suitable for analysis however so the actual sample sizes may differ, due to for instance background noise (see table 2.1 for a list of all populations and sample sizes in this analysis). Songs were in (or down sampled to) 22.05 kHz. Because we had relatively few suitable songs from Australian regions (Alice Springs and Northern Victoria) we decided to merge these to one population. Moreover, because of the high level of dispersion in wild zebra finches, there are no clear dialects found in wild zebra finch song (Zann 1996), nor large genetic differences between wild populations (Forstmeier *et al*. 2007). For some of our analyses populations were grouped in three continent groups, according to geographical proximity. We arbitrarily grouped the Auckland population from New Zealand with the wild population sample from Australia.

Table 2.1. List of populations with amount of syntax scores (σ).

lab population	continent*	sample size	σ	highest k	bootstrap p-value	sign
ALL	-	189	-0.016	8	p> 0.5	
Auckland	Australia	9	0.108	4	p<0.025	*
Bielefeld	Europe	18	0.082	4	p>0.025	
Berlin	Europe	15	0.169	6	p<0.0025	*
New York (CU)	N. America	14	0.018	3	p>0.025	
New York (HC)	N. America	15	0.146	10	p<0.025	*
Leiden	Europe	15	0.050	6	p>0.025	
Australia wild	Australia	17	0.128	4	p<0.025	*
Montreal (MG)	N. America	15	0.121	10	p<0.025	*
St Andrews	Europe	14	0.102	9	p>0.025	
St Etienne	Europe	15	0.138	2	p<0.025	*
San Fransisco	N. America	15	0.178	2	p<0.0025	*
Seewiesen (MPI)	Europe	13	0.061	7	p>0.025	
Williamstown (WC)	N. America	14	0.096	7	p<0.0025	*

* 'continent' refers to grouped in terms of geographical distances. CU=Columbia University, HC = Hunter College, MG = McGill University, MPI = Max Plank Institute for Ornithology, WC = Williams College

Song analysis by computer software: Luscinia

Zebra finch song is known for it's relatively difficult segmentation and annotation in element types and/or syllables. Except for Lachlan *et al*. (2010) all previously mentioned articles have been based on visual inspection for assessing element categorization. Even though the use of several observers can reduce the variability of visual classification (Jones *et al.* 2001), such studies are limited in the sample size of songs they can analyze, and the degree to which quantitative measures of inter-element similarity can be provided (as opposed to same/different binary scoring). It is therefore difficult to statistically address questions of phonological and syntactical organization without computational methods.

Recently, several software packages (e.g. Sound Analysis Pro, Tschernikovski *et al*. 2000, Luscinia, Lachlan *et al*. 2010) have been developed that allow the acoustic analysis of large datasets of birdsongs. By using computational algorithms to compare songs, quantitative comparisons between large numbers of elements can be made, with little or no observer bias. We used the software package Luscinia (http://luscinia.sourceforge.net) for acoustic and statistical analysis. In Luscinia, measures of several acoustic parameters are taken at each spectrogram time slice throughout each element. Sound files were filtered in Luscinia with a 100 Hz high pass filter. Luscinia then uses an implementation of Dynamic Time Warping (DTW) to compare elements based on these time-series vectors. (See the SI for the Luscinia settings and parameters chosen for this study.) The output of this comparison is a matrix of the dissimilarities between each pair of elements in the dataset. This matrix can be ordinated into Euclidean space using nonmetric multidimensional scaling. The resulting principal components can then serve as the basis for cluster analyses in statistical software packages like R (and open-source package for statistical analysis available from www.r-project.org). With this species-wide clustering solution it is possible to identify element clusters specific to populations or universal to all populations.

Motif selection

As shown in fig 2.1, zebra finch songs consist of motifs, preceded by a number of introductory elements. Repeated motifs are called a song bout. Motifs can vary slightly within birds. We selected the predominant motif of each bird for analysis (i.e. the motif with the highest probability for that bird (Sturdy *et al*. 1999a; Helekar *et al*. 2000). Introductory notes were excluded unless they recurred in motifs later in the bout. Motifs were selected by two observers (StH and CvH). Each observer selected half of the motifs for each population reducing the possible effect of observer bias. For a subset of the songs both observers selected the motif in order to test reliability. For this reliability estimate, a subset of the motifs was selected by two observers. The percentage of motifs that was the same for both observers was used as a measure of reliability. This reliability between the observers was relatively low: 0.60, as many times there was not one clear dominant motif, but several that occurred relatively frequent. Therefore, the songs that were ambiguous were re-examined by both observers and the motif for which consensus was reached was selected.

Element segmentation

Throughout the years of zebra finch song research, multiple methods of element segmentation have been published (e.g. Jones *et al*. 1996; Scharff and Nottebohm 1991; Zann 1993a and b; Sturdy *et al.* 1999a; Lachlan *et al*. 2010). These are not all in agreement. In the present study we segmented motifs into elements based on the criteria of silent gaps and/or abrupt changes in frequency and amplitude. The segmentation was performed manually by StH and CvH, using Luscinia, after several years of experience with zebra finch song analysis and multiple training rounds on other songs. Each observer performed element segmentation on half of the motifs from each population reducing the effect of observer bias. For an estimate of reliability a subset of the data was segmented by both observers. Agreement was calculated as the percentages of elements that were segmented similarly by the two observers. Since the total amount of elements was slightly different for each observer, two percentages were calculated; one for each observers' total number of elements per motif (83 % and 89 %). The average agreement between observers was 86 %.

Phonology

Cluster analysis

A cluster analysis was performed with Mclust (package 'mclust' was built under R version 2.12.2) (Fraley and Raftery 2002) within R (version 2.13.0, available at http://www.r-project.org) on the output from Luscinia (the first ten PCO values which explained more than 92% of the variation in the data set). Mclust clusters data using Gaussian Mixture Modeling. This approach allows for clusters of varying size and shape to be discovered (Fraley and Raftery 1998). Mclust then uses the Bayesian Information Criterion approach to determine which model best fits the data, allowing a statistical assessment of the number of clusters present.

Having placed elements into clusters, further statistical phonological analyses were performed on the zebra finch songs annotated according to the element types calculated by Mclust.

Relative position of element types within a song

Some element types may be restricted to certain positions in the song. For instance the songs of chaffinces (*Fringilla coelebs*) show these patterns (Slater and

Ince 1979). The relative position of elements in a motif was estimated by ranking each element according to its position in the song (low numbers for elements early in the song and higher numbers for those at the end). Because songs differ in total number of elements, a relative measure of position was calculated by dividing the position number by the total number of elements per song. For example: the 8th element in a 10-element song has a relative position of '0.8'. A relative position was determined for each element in each song. The mean relative position was calculated for each cluster of elements (as classified by the Mclust algorithm in R).

Phonological syntax

Species-wide syntax analysis

The amount of structure, or syntax, was measured with a Sliding Window Match Length (SWML) entropy estimator that analyses the sequences of elements in the zebra finch songs (Suzuki *et al*. 2006). 'Entropy', in this context, is a measure developed in the field of information theory to describe the unpredictability in a signal. This theory has been applied before to find patterns in animal vocalizations, for instance by Lemon and Chatfield (1973) in rose-breasted grosbeaks (*Pheucticus ludovicianus*), by Suzuki *et al*. (2006) in humpback whales (*Megaptera noveangliae*) and by Briefer *at al.* (2010) in skylarks (*Alauda arvensis*).

The higher the information entropy, the lower the degree of structure or constraints in the data. For instance, a random sequence of different element transitions results in a very high entropy value. In contrast, messages with lexicographical, grammatical and contextual constraints result in a lower entropy value as not all combinations of elements are present in the message (Suzuki *et al* 2006).

One limitation with entropy-based measures of syntax is that they require constituent elements to be placed into discrete categories. Different categorizations of elements may result in very different entropy estimates. A solution to this problem was proposed by Lachlan *et al*. (in prep). This method searches for the partitioning of elements that minimizes entropy (that is maximizes syntax). There are two steps in this method. In the first, elements are assigned to clusters based on their acoustic similarity (as determined from the DTW comparison, above) to a predetermined number of *k* cluster centroids. In the second step, entropy is estimated using the first order Markov estimator for the clustered data. The algorithm proceeds by searching for centroids that minimize the entropy estimator in a similar way to the k-medoids algorithm (from which this algorithm is adapted).

Once the partitioning with most structure had been identified, we recalculated entropy for that partitioning using the SWML estimator. The benefits of this type of entropy estimator are that it is applicable to a relatively broad range of data, as it has less a-prior data assumptions than other syntax inducing algorithms. Also, it is relatively robust to departures from these assumptions. And lastly, even if the sample sequence is not long enough to achieve asymptotic convergence, the resulting entropy estimates are an upper bound on the source entropy (Suzuki *et al.* 2006). Therefore, even with relatively small sample sizes this method can estimate the amount of structure present in zebra finch song reliably. It is, however, too slow to use during the clustering part of the algorithm (above). We set the window length for this algorithm to be the length of the individual motifs. We then rescaled entropy as 'redundancy', calculated as $(H_{baseline}-H_{swml})/H_{baseline}$. We used $H_{baseline}$, the entropy calculated from randomly sequenced sequences instead of the maximum entropy (H_{max}) that Suzuki *et al.* (2006) used as $H_{baseline}$ turned out to be a more stable parameter. Our redundancy estimates then served as our measures of syntactic structure. Redundancy is a way of scaling entropy were '0' means that element transitions are random (like randomly shuffling the sequences of elements in each song) and '1' means that sequences are entirely predictable and non-variable. We estimated confidence limits around our estimates using a bootstrapping technique (Suzuki *et al*. 2006). We estimated redundancy for values of *k* between 2 and 10 and selected the highest redundancy value as our estimate of syntactical structure for the particular level of analysis (species-wide, per population or per continent).

Population level syntax analysis

The individual redundancy scores per population were calculated and bootstrapped to arrive at confidence intervals around these scores to indicate presence or absence of structural constraints, or redundancy (σ, 'rho') per population.

The redundancy analysis returns one value per population, so in order to compare the populations with each other for their degree of syntactical structure, a deviation score for each individual in each population was calculated and analyzed in an ANOVA.

The deviation (calculated from the SWML distances, converted to PCO values) is a measure for how different song syntax is between one song sequence and its population average (centroid), an adaptation of the method described for variation tests by Anderson (2006) and Anderson *et al*. (2006).If songs are on average further away from the centroid, they also on average vary more from each other, and hence have a higher entropy (and are therefore less structured or constrained in terms of syntax).

Continent level syntax analysis

To calculate the amount of syntax in each continent, pairwise σ scores for within-continent populations were calculated instead of σ scores per continent. The latter scores are less suitable in this context because the Australian continent contained data of only two populations (one wild population from Australia and 1 laboratory population from Auckland, New Zealand). The other populations contain 6 (North America) and 5 populations (Europe) and the σ is influenced by the number of populations in the comparison. We therefore calculated pairwise σ scores between populations within continents to estimate the amount of syntax within continents and bootstrapped the results to calculate confidence intervals. We then averaged over the pairs within continents to arrive at an average and confidence interval per continent.

RESULTS

Phonology

To cluster all elements according to acoustic parameters we measured them in Luscinia and performed a DTW analysis which was followed by an NMDS and subsequent PCO analysis based on 10 components. These 10 components explained 92% of the variation (Kruskal stress value of 0.051, indicating a good fit (Ozturk *et al.* 1999).

The Mclust algorithm calculated an optimal model of 11 clusters (see fig. 2.2a, b and c) in a VEV configuration (Ellipsoidal, variable volumed, equal shaped and variably oriented) and a BIC value of 8821.2. The next best fitting models had BIC values of 8820.8 with 14 clusters and 8777.1 with 13 clusters respectively. We chose the 11-cluster model as this one had the highest BIC and it is the most parsimonious model where the number of clusters is concerned. To estimate the overall tendency for the data to cluster, we compared the one cluster solution BICvalue (-2283.4) with the value Mclust gives for the optimal clustering model. The large difference between the two indicates a very high propensity of the data to fall into clusters.

To give an example for each of our clusters, we selected the first three elements from the densest area of each cluster to characterize the element type. If the first three elements would not give a conclusive characterization, we analyzed four elements (in the case of cluster 3 and 8). For labeling of the element clusters, see fig. 2.3.

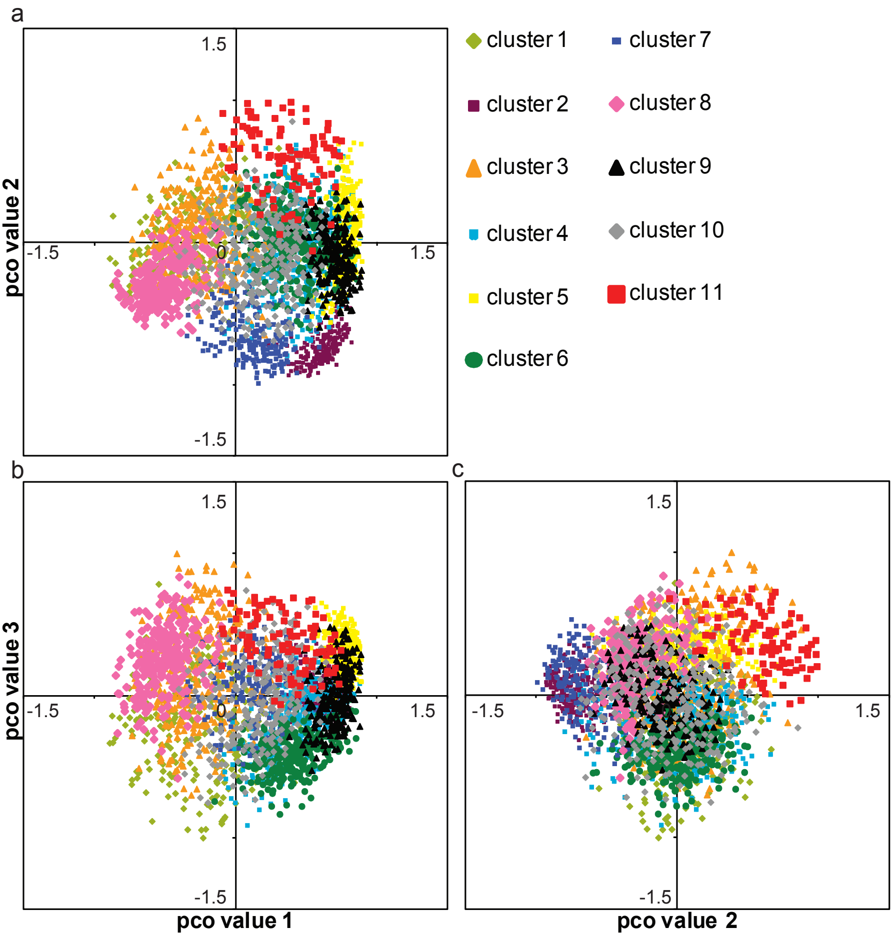

Fig 2.2. Clustering graph of the data from all populations together into 11 element types. a) PCO value 1 versus 2. **b)** PCO value 1 versus 3. **c)** PCO value 2 versus 3. Each datapoint is an element. Different colours (and shapes) represent the different element types (clusters as modeled by Mclust).

The distribution of the 11 clusters per laboratory, per continent and species-wide is shown in fig. 2.3. All clusters occur in every population, indicating that all element types occur in all populations and therefore that there are no population specific element types. Yet, we found that the elements from the different clusters are not distributed evenly over the populations (X^2 permutation test in which individuals were permuted between populations with 10000 permutations, $X^2 = 441.8$ $p < 0.001$, Cramér's effect size 0.13).

We also found that continents differed in element type distribution (X^2 permutation test in which individuals were permuted between continents with 10000 permutations, $X^2=162.9$, $p <0.01$, Cramér's effect size 0.17).

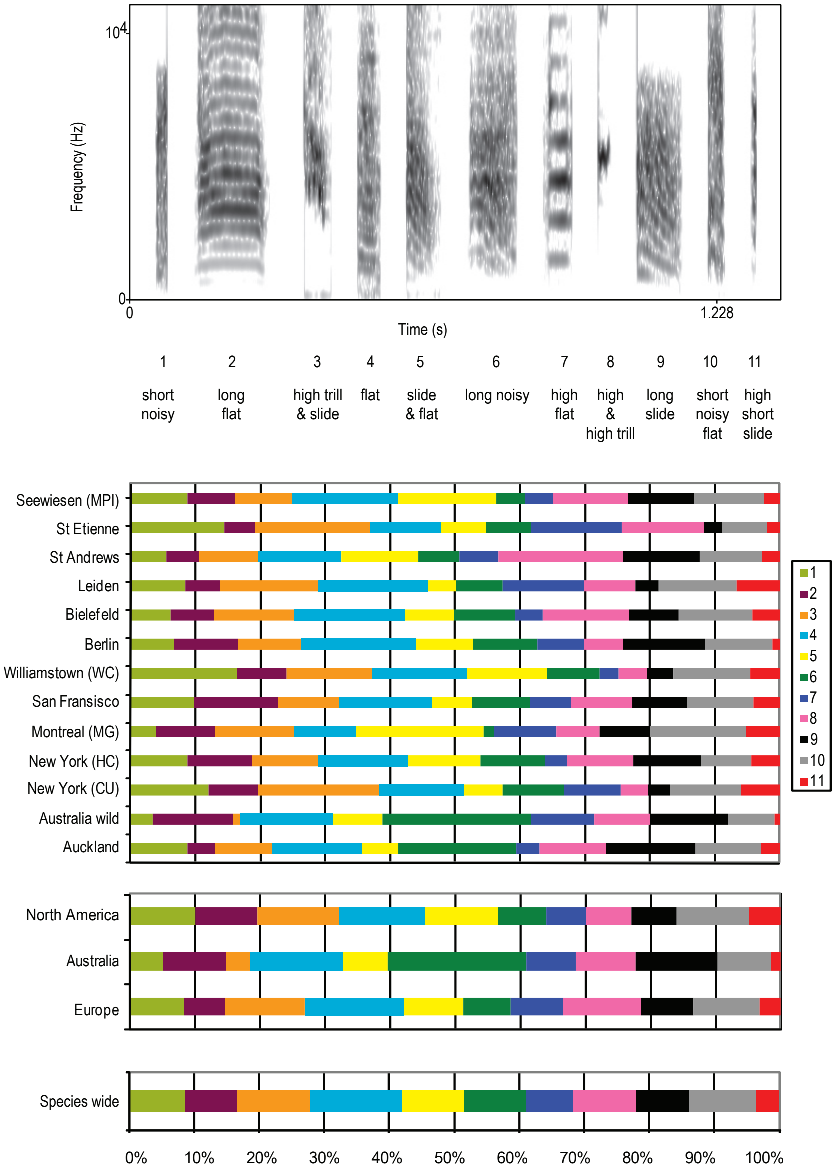

Fig 2.3. Element types and their distribution at population, continent and species level. The top panel provides representative examples of the element types found in the Mclust clustering analysis. Numbers indicate individual element types (clusters). Lower panels show element type distributions in percentages per population, continent and species-wide level.

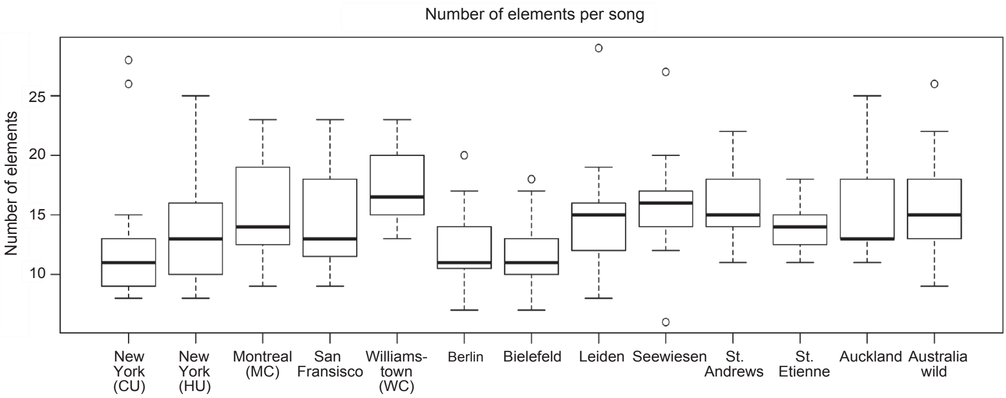

Fig 2.4. Boxplot distribution of the number of elements per song per population.

Number of elements per song

There was variation in the number of elements per song between the different populations, see fig. 2.4. (F=2.344, deletion p = 0.008, based on 188 df, with a glm model with quasi-poisson link). However, when aggregated over continents, there were no differences in element number per song (F=1.736, deletion p > 0.05, based on 188 df, with a glm model with quasi-poisson link).

Relative position of elements within a song

The mean relative position of each of the 11 element types is presented/shown in fig. 2.5. The overall Kruskal-Wallis test was significant (K-W X^2=166.84, df=10, p <0.001 and therefore the element types are not occurring evenly over the positions in the song. Further testing showed that on a species-wide level clusters 2 (two-sample Wilcoxon test, W=158762, p <0.001, and 7 (two-sample Wilcoxon test W=198835, p <0.001) occurred in the end region of the song (see fig. 2.5). (All tests were still significant after Bonferroni correction for multiple testing). Roughly the same distribution pattern can be observed on continent level: For all continents, the overall K-W tests were significant: Australia: X^2= 32.4, df = 10, p < 0.001; North America: K-W test X^2= 125.3, df = 10, p < 0.001; Europe: K-W test X^2= 52.8, df = 10, p < 0.001. After post-hoc tests, we found similar patterns compared to the species-wide pattern, except for Australia, where cluster 9, but not cluster 7 occurred relatively at the end (two-sample Wilcoxon test W= 6847, p = 0.006, still significant after Bonferroni correction).

Phonological syntax

Species-wide level syntax

After analyzing the songs with the k-medoids/Markov chain modeling and SWML estimator algorithm in Luscinia, we found a mean redundancy (σ, amount of structural constraints) that did not significantly differ from 0 (-0.016

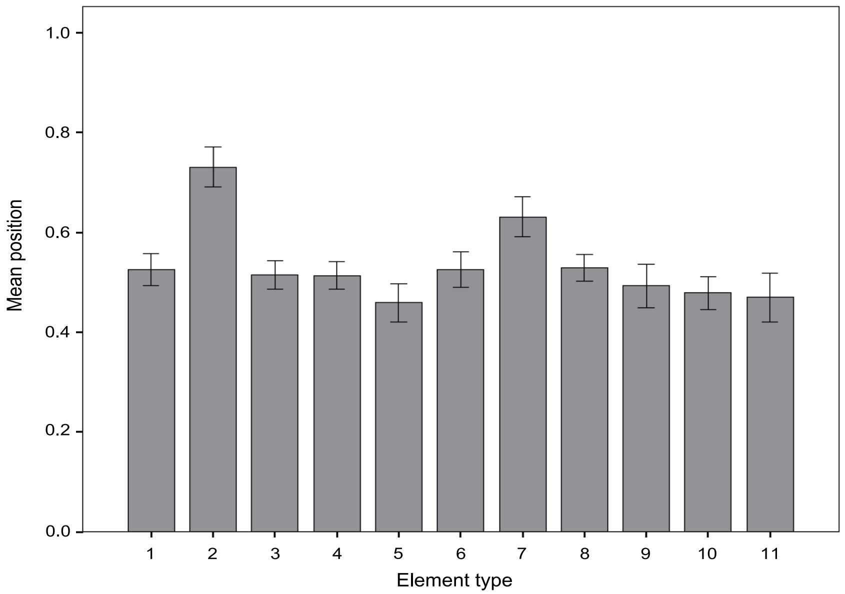

Fig 2.5. Relative element position on species-wide level. A mean position of 0.5 indicates thath the element type does not particularly occur at the beginning nor at the end of a song. A mean position of 0.7 means that that element type occurs relatively often towards the end of the song. Error bars represent 95% CI.

with maximum redundancy at k= 8 syntactical clusters CI 95%: -0.065 to 0.030). This implies that there is no evidence for syntactic structure, in terms of sequences of specific element types, on a species-wide level in zebra finch song.

Per population syntax

When the data from each individual population were analyzed separately for the amount of structure (redundancy), we found that the bootstrapped estimate of , averaged over all populations was 0.109. This is higher than when all populations were analyzed together on the species-wide level. For eight of the populations, there was significant evidence of syntax (see table 2.1 with pop mean σ's, k= nr of clusters and bootstrap p-value. Estimates of σ were not influenced by sample size in any detectable way when comparing between populations, indicating that these sample sizes were adequate (see fig. SI 2.1 in the SI).

We compared the syntactical structuring between the different populations by calculating the deviations, but there were no significant differences found (ANOVA, df 188, F= 1.35, p-value 0.19), so there were no significant differences between the lab populations in the amount and variability of syntax in the songs of that population.

Per continent syntax

Estimating continent-wide syntactical structure was complicated by the relatively low number of Australian populations (two: 1 wild, 1 lab from Auckland) compared to the others (five and six). Based on the population level analysis (above), this would be expected to artificially boost the estimate from Australia compared to the other continents. To avoid this effect we therefore made pairwise comparisons between all possible pairs populations within each continent and calculated deviation scores. The mean deviation values averaged over the pairs of populations in these continents were: 0.047 (North America, with CI 95%: -0.054 to 0.140); 0.083 (Australia, with CI 95%: -0.012 to 0.175), and 0.036 (Europe, with CI 95%: -0.052 to 0.122). However, none of these scores was significantly different from 0, indicating that there is no evidence for structure on a continent level and therefore also no variation between continents.

GENERAL DISCUSSION

Our computational analysis of zebra finch song from 13 populations indicates that in terms of phonology, there is evidence for clustering of elements into 11 types on a species-wide scale. All element types occur in all populations but the distribution of element types differs significantly between populations as well as continents. In terms of syntax, or structural constraints found in the sequences of elements in zebra finch song there is variation in the amount of syntax on population level, but these effects are absent when looking at continent and species-wide scale (all results from this study are summarized in table 2.2).

Table 2.2. Results overview

	phonology	**tot nr of elements**	**relative position**	**phonological syntax**	
level of analysis	**element types**			**amount of syntax (ρ)**	**deviation**
species-wide	11 clusters	-	2 and 7 in end region	0	-
continent	variation in the distribution of these 11 clusters	no variation	2 (Aus,Eur, N-Am), 7 (Eur, N-Am), 9 (Aus) in end region	0	-
population	variation in the distribution of these 11 clusters	variation	-	8/13>0	not sign

Phonology

The 11 element categories as obtained by the clustering algorithm analysis of all 13 zebra finch populations together is different from earlier findings using a comparable analysis, but data from three populations only (Lachlan *et al*. 2010). That analysis resulted in two categories; high notes and all low frequency elements together. The difference may be explained by the number of populations used. The difference in clustering method however, may also account for some of the differences, since the Mclust method used here also takes into account different cluster shapes and sizes, whereas the simple agglomerative clustering method used in the previous study assumed perfect spherical clusters. Other studies have described different note types as well, using visual inspection of spectrograms of laboratory birds' songs (Price 1979; Williams and Staples 1992; Sturdy *et al*. 1999a), or songs from wild individuals (Zann 1993a and b). Zann reported 14 element types, Sturdy 5 types and Price also 5 types. There is some overlap in categories described previously and the ones found in the present study. For instance high notes and slides are describes as separate categories by Zann (1993a and b) and Sturdy *et al*. (1999a). This is also valid for the present study, however multiple categories of slides and stacks are found. Sturdy *et al.*'s study including four laboratory populations also found differences in distribution of element types between populations but every category was found in all populations (Sturdy *et al*. 1999a). This seemingly corroborates our present findings. It should be noted however, that the classification by Sturdy *et al.* was aimed at providing generalizable information about zebra finch song. Thus Sturdy *et al.* probably deliberately classified elements (or note types) into more universally applicable categories.

Differences in classification methods between studies make a more direct comparison with the present results difficult. For instance the previous authors used silence as a segmentation criterion, and therefore their classification includes larger units indicated as 'combination elements'. In our study these combination elements are segmented into multiple elements as we also included abrupt changes in frequency as a segmentation criterion. At this point it is not entirely clear what level of representation is that birds use themselves while processing songs. Ten Cate and Slater (1991) as well as Williams and Staples (1992) reported that songs are copied mostly as chunks separated by silent intervals, which may include several elements and/or syllables. The same studies, however also demonstrated that elements and syllables within chunks could also be copied separately, indicating a more detailed representation. Therefore, in the present study we chose an analysis on the element level.

None of the 11 element clusters found in the present study is specific to any of the populations (see fig. 2.3) and each cluster appeared in each population.

The species-wide distribution of element clusters does show significant variation between populations, however. For instance, cluster 11 seems more consistent in presence among populations compared to cluster 3, which appears to show much more variation in abundance. Although perception and production might go in parallel, it is yet unknown whether the zebra finches would perceive the same 11 clusters we obtained as separate perceptual categories. Since the distribution of categories differs between populations in the present study, it is also possible that category boundaries or even the clustering itself might differ across populations (Prather *et al*. 2009). Using clustering methods similar to those in the present study but rather on population level, could give insight in the position of these boundaries. However, for the present study this was not possible due to constraints in sample size. Variation in distribution of element clusters is also found when populations are grouped per continent, implying that there is some continent specific phonology (see fig. 2.3). The effect sizes from both the population and continent analysis are rather similar (0.13 and 0.17 respectively).

The study by Forstmeier *et al*. showed genetic differentiation between zebra finches in different continents, in addition to considerable variation within continents (Forstmeier *et al*. 2007). When we compare our phonological results to the genetic relatedness, as provided by the unrooted dendrogram from Forstmeier *et al*. (2007), we found a slightly different pattern, namely that the variation between continents was more or less equal to the variation between populations, instead of continent variation being larger than population variation, indicating that larger genetic differentiation is not paralleled by larger song differentiation. This is a pattern that might be expected when the prime factor for song differentiation is cultural rather than genetic differentiation, as cultural differentiation might be driven by other factors than genetic ones.

To analyze this in more detail, one would also need information on the cross-laboratory exchange history of birds. On the other hand, the fact that similar element types exist among populations that have been separated for several generations suggests that the cultural variation might to a certain extent be constrained by perceptual or learning biases. That certain constraints are present that shape the song into a certain direction has also been indicated by an intriguing experiment by Feher *et al*. (2009). They used birds that were raised without exposure to song, and therefore developed aberrant isolate song and used these as tutors for the next generation. After approximately three generations the song features were already similar to wild-type song. Moreover, a recent study in our lab suggests that biases for acoustic features common to zebra finch song may be present in juvenile birds before they learn song (Ter Haar *et al*. in prep).

Total number of elements per song

There is significant variation in the total number of elements per song between the different lab populations (see fig. 2.4), but this was not the case when aggregated over continents. Slater and Clayton (1991) did not find such a difference when analyzing three populations. However, they analyzed the number of *different* elements per individual song. Our analysis is not suited for a direct comparison as it is based on element *types*: two elements in a song may belong to the same basic type, but still be noticeably different. We also did not find clear evidence for the occurrence of repeats, as was found by Helekar *et al*. (2000 and 2003). It would be interesting to further analyze the dataset for within song immediate repeats and subsequently whether these can be linked to specific element types.

Relative position of element types within a song

First, we examined whether there are associations between element type and relative position in the song. When all lab populations are averaged, the long flat element type from cluster 2 (long flat), and to a lesser extend also cluster 7 (high flat), are positioned towards the end of the song (see fig. 2.5). The same pattern can be observed for North America and Europe. Interestingly, in Australia, also cluster 9 (long slide), and not cluster 7, has a tendency to occur in the end region of the song (see fig. SI 2.2). An explanation why this did not lead to a higher σ in the redundancy syntax analysis might be that the long flat element do not necessarily occur only exactly as the last element of the song, but on average in the end region (mean position 0.7) and can be flanked by any type of other element. The link between long flat notes and the end region of the song has also been observed by other researchers (Zann 1993 a, b and 1996).

Phonological syntax

On a species-wide and continent level, we did not find evidence for species-wide constraints in the sequences of zebra finch song. The estimates of redundancy found for zebra finches in this study were, for example, much lower than a recent study of chaffinches, which found population-level redundancy values of approximately 0.5 (Lachlan *et al*. in prep). Similar to our results, Lachlan *et al*. (2010) found no evidence for clear structural constraints in zebra finches based on data from three lab populations (from different continents).

Nevertheless, on population level we did find evidence for the presence of syntactical constraints in 8 of the 13 populations. So, in a number of populations we found restrictions in what kind of song sequences the local zebra finches sing, while in others there is no evidence for such restrictions or syntax. At first sight this might seem to conflict with the finding that on a species-wide scale, we found no evidence for such constraints in zebra finch song. This is not due to small sample sizes, which potentially might affect the SWML estimator (Suzuki *et al*. 2006). As shown in fig. 2.1 of the SI, the estimates of σ were not influenced by sample size in any detectable way when comparing between populations. On the other hand, we found a significant difference in the deviations when aggregated for all populations compared to the individual deviations calculated for each population separately (paired t test, t= 13.35, p <0.001). Therefore, although there was syntactical structure within populations, it varied between populations. Thus when the populations were aggregated and analyzed together, there was no evidence for species-wide structure.

The most likely explanation is that there might be an effect of tutor sharing leading to frequently occurring sequences in particular populations. Zebra finches can incorporate chunks (short sequences of elements) from their tutor(s) in their song (ten Cate and Slater 1991) and can also from learn other males nearby (Williams and Staples 1992). The most obvious way that two individuals can share elements is if they are siblings. Although the aim was to keep the number of brothers in the laboratory datasets as minimal as possible, this was not always possible to avoid, especially when birds are allowed to breed in aviaries and the paternity cannot be established easily. We could not control whether males shared ancestors in earlier generations, such as grandfathers. Nevertheless, such a process would resemble the formation of dialects known from other song birds and human literature (Marler and Tamura 1964; Tserdanalis and Wong 2004, respectively).

What is important to keep in mind here is that is hard to quantify syntactical structure; different methods make different assumptions, and consequently capture different aspects of syntactical structure. It is possible that we missed patterns on a higher level such as syllable- and chunk sequences or nonadjacent dependencies. Furthermore, as a first effort, we chose to include one motif per individual, but it might be interesting to take into account the intra-individual variation in future studies as well.

Domestication

The differences in the relative frequencies of element types and syntax that we observed between different populations might be partially due to domestication. Effects of domestication in songbirds have been observed in a closely related species, the Bengalese finch (Honda and Okanoya 1999). This species has a longer history of domestication (approximately 260 years, compared to approximately 150 years in zebra finches estimated by Sossinka, 1970 and Rogers, 1979). It has been shown that the song of domesticated Bengalese finches is less constrained and has different acoustic characteristics compared to that of wild strain birds (Honda and Okanoya 1999).

It might be that after a longer period of domestication, zebra finch populations show more phonological diversity than they do now and that the current commonalities are partly a result of reliable cultural transmission. However, the history of zebra finch domestication, if any, before 150 years ago is mostly unknown (Sossinka 1970) so whether the difference between Bengalese finches and zebra finches is due to a difference in duration of domestication remains an open question.

In a domesticated species such as the zebra finch, various factors may give rise to differences between populations, such as founder/common tutor effects in small populations that can give rise to random drift in song features. Moreover, breeders determine the number and size of generations and may select for specific traits, such as bird size and clutch size. Also the influx of new birds from other populations may affect the outcome. In case of aviary breeding, female choice for specific male (song) traits may play a role (see Riebel 2009 for a review on female preference in zebra finches). However, it remains difficult to disentangle the relative effects between cultural transmission, biological evolution and domestication on the evolution of song. Nevertheless, the methods in the current study present a useful set of tools to investigate such issues in more detail.

Taken together, our findings indicate that data from one population may not always be representative for zebra finches in general, and hence generalizations should be done with caution.

Comparison with human language

Songbirds are often used as a model for studying vocal learning and language or speech. The current findings may offer some insight in how biological and cultural evolution may relate to difference in sound patterns across populations worldwide. A direct comparison with language variation among populations of humans is difficult. In terms of phonology usually one language or dialect has a different phoneme inventory then another, but all individuals within a population make use of the same inventory. This is different in zebra finch populations, first of all because individual differences between birds in element use are abundant (thus only a subset of the elements of 11 clusters, are present in all individual birds, but all element clusters are present in all populations).

Second, the present findings suggest that all phoneme categories are present in all populations. However the different distribution of elements may still tell something about cultural evolution, since some categories seem more flexible than others. This is similar to the results of some studies on language change that suggest that some phonemes are more prone to change than others (Although the cause of such change is a subject of debate (Moreton 2008)).

Additionally, language and speech research shows that some aspects of languages are (initially) universal in both perception and production, indicating biological constraints on the possible variation of sounds (Jackobson 1941/68). It would be interesting to see in a longitudinal study whether the more flexible element categories found in the present study indeed show more change over time than the less flexible ones.

CONCLUSION

By using a computational approach, we found 11 element types occurring among all 13 populations. Despite these commonalities there is considerable variation between populations in the distribution of element type frequencies. For syntax, the only commonality in terms of sequences of elements seems to be the position of long flat elements towards the end of the song. However, 8 out of 13 populations had a significant amount of structural constraints in their songs, although these constraints were absent on a species-wide and continent level.

These results shed light on the apparent variation in song characteristics between populations of a widely used model species for language evolution and vocal learning. The findings may be important for interpretation of results from different populations and other (bird) species used in experimental research. Moreover, they are suggestive of cultural differentiation being at least as important as genetic diversification processes in causing large scale changes in song characteristics.

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SUPPLEMENTARY INFORMATION

Luscinia Settings list

Settings and parameters we included in the Luscinia analysis were: frame length 7.5; time step 0.5; weight by amplitude; log transform frequencies; time, fundamental frequency, fundamental frequency change and harmonicity; compression factor 0.25; SD ratio 0.5.

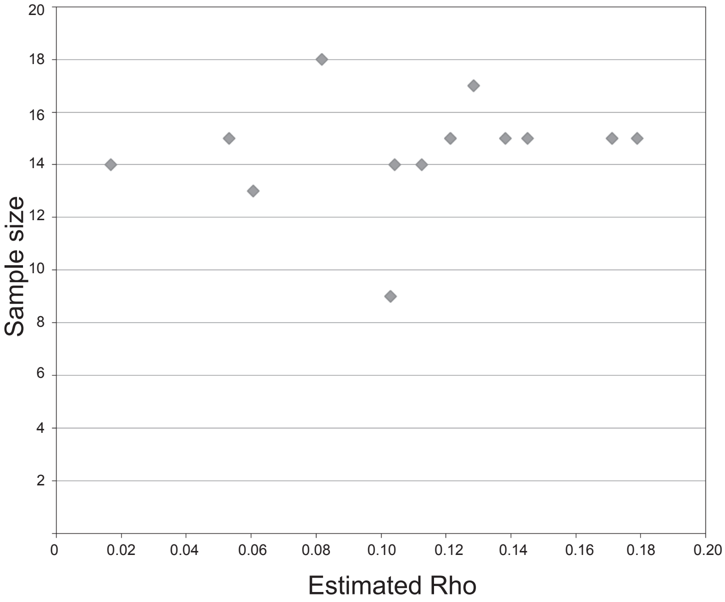

Fig SI 2.1. No relationship between population size and estimated σ (rho).

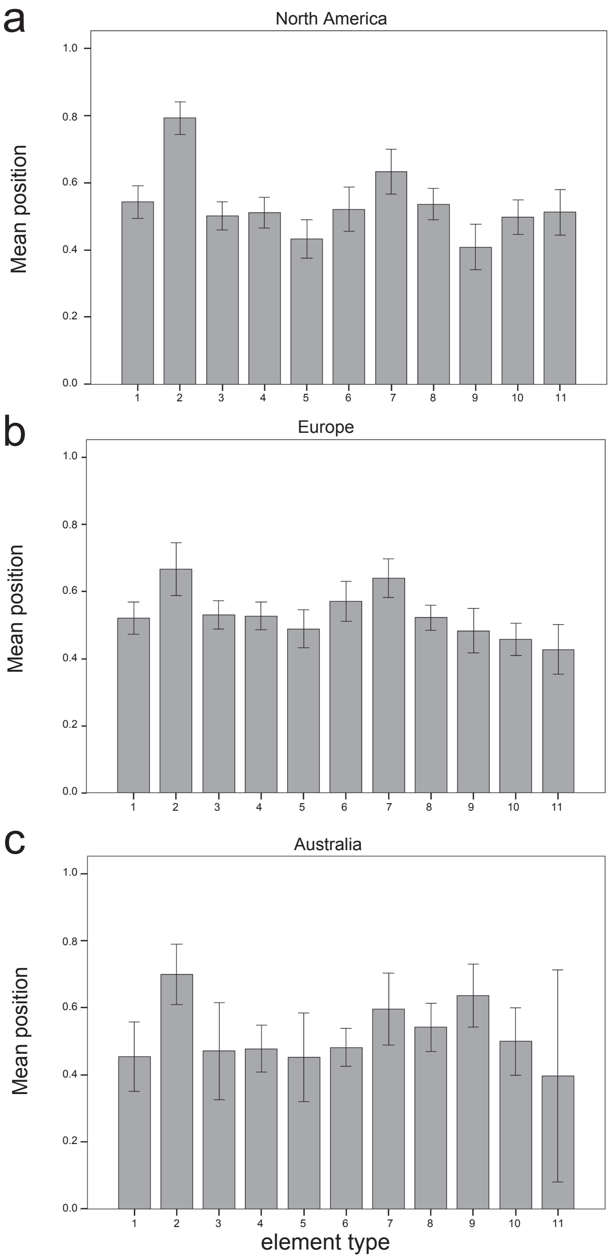

Fig. SI 2.2. Relative element position in the song on continent level. Error bars represent 95% CI. **a)** North America. **b)** Europe. **c)** Australia.

Chapter 3

Simple rules can explain discrimination of putative recursive syntactic structures by songbirds: A case study on zebra finches

C.A.A. van Heijningen, J. de Visser, W. Zuidema and C. ten Cate

ABSTRACT

According to a controversial hypothesis, a characteristic unique to human language is recursion. Contradicting this hypothesis, it has been claimed that the starling, one of the two animal species tested for this ability to date, is able to distinguish acoustic stimuli based on the presence or absence of a center-embedded recursive structure. In our experiment we show that another songbird species, the zebra finch, can also discriminate between artificial song stimuli with these structures. Zebra finches are able to generalize this to new songs constructed using novel elements belonging to the same categories, similar to starlings. However, to demonstrate that this is based on the ability to detect the putative recursive structure it is critical to test whether the birds can also distinguish songs with the same structure consisting of elements belonging to novel, unfamiliar categories. We performed this test and show that seven out of eight zebra finches failed it. This suggests that the acquired discrimination was based on phonetic rather than syntactic generalization. The eighth bird, however, must have used more abstract, structural, cues. Nevertheless, further probe testing showed that the results of this bird, as well as those of others, could be explained by simpler rules than recursive ones. Although our study casts doubts on whether the rules used by starlings and zebra finches really provide evidence for the ability to detect recursion as present in 'context-free' syntax, it also provides the first evidence for abstract learning of vocal structure in a songbird.

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Chapter 3

INTRODUCTION

Studying how different features of human language relate to vocal and cognitive abilities in other animals can provide insights into the evolution of the language faculty. A key feature of language is its syntax: the system of rules that govern the construction of sentences by combining smaller elements such as words. It is broadly accepted that the rules that structure animal vocalizations are much simpler than those that structure human language, but it is hard to pinpoint the critical difference. In an influential, but controversial paper, Hauser, Chomsky and Fitch (HCF) (2002a) formulated various possible hypotheses, one of them stating that "recursion is the only uniquely human component of the faculty of language" (see also below).

Recursion is typically defined as the embedding of a unit of a particular category inside a larger unit *of the same category*. In the context of language this enables so called 'discrete infinity', whereby an unbounded number of different sentences can be constructed from a limited number of different words. For instance, the sentence 'the woman saw', can be embedded in 'the man left', which results in: 'the man the woman saw left'. When we represent the noun phrases with A's and the verbs with B's, this sentence follows an AABB structure, with an AB 'recursively' embedded inside another AB. (But see below; the Discussion and Corballis (2007a) for the concerns about the actual definition of linguistic recursion; its relation with mathematical recursion and its usage by humans).

A formalism often used to describe such center-embedded recursion is that of context-free grammars, which form a distinct level in a popular complexity metric known as the Chomsky Hierarchy; the A^nB^n structure (requiring an arbitrary but equal number of A's and B's) is an example of a structure that cannot be generated by grammars on lower levels on the Chomsky Hierarchy. This is because it requires an unbounded memory to make sure as many B's are produced as there were A's in a string. No such memory is required to produce sentences that follow the $(AB)^n$ structure (such as ABAB or ABABABAB) in which the next AB is concatenated to the previous AB; This is an example of a structure that can be generated by a Finite State Grammar.

It is widely accepted that finite-state grammars are insufficient for describing the syntax of human language (Chomsky 1957). In contrast, there is no evidence to date showing that animal vocalizations are also beyond the power of finite-state grammars. However, there is a debate about whether animals have the cognitive ability to *detect* more complex syntactic rules in artificially constructed stimuli. Until now, two studies have addressed this issue in other species, one on non-human primates and one on a songbird species.

Fitch and Hauser (2004) trained and tested humans and cotton-top tamarins (*Saguinus oedipus*) in a familarization/discrimination paradigm for the ability to distinguish between the above mentioned types of structures. The auditory stimuli consisted of (human) consonant-vowel (CV) syllables spoken by males and females organized in A^nB^n or $(AB)^n$ structures (with n=2 or n=3). While humans were sensitive to a change in structure in either direction, the tamarins did not seem to notice a transition from the A^nB^n to the $(AB)^n$ structure. Fitch and Hauser interpreted this as evidence that tamarins are unable to learn context-free languages and are limited to the less complex, non-hierarchical finite-state languages such as the ABAB structure.

The experiments gave rise to a lively debate, ranging from questions such as whether the stimuli really represented a suitable test of the ability to detect recursive structures, to whether animals as well as humans can 'solve' the task by using less complex, non-recursive strategies (Kochanski 2004; Perruchet and Rey 2005; Zuidema 2005; Bahlmann *et al*. 2006; Liberman 2006; Corballis 2007a and b; De Vries *et al*. 2008; Hochmann *et al*. 2008). From a methodological perspective, one concern about the study is that non-conspecific, human speech stimuli were used to test the tamarins, while this species (like most primate species) also has a limited vocal repertoire and lacks the ability for vocal learning. The failure of the tamarins might thus be explained by more basic features of their perceptual and learning abilities rather than the ability to represent and process syntactic structure. A stronger test for the human uniqueness of syntax comes from comparative studies with animals, such as songbirds, showing vocal learning and complex vocalizations, in particular from studies where the stimuli consist of units present in their natural vocalizations. Such an experiment was carried out in the starling (*Sturnus vulgaris*) (Gentner *et al*. 2006). Starlings are songbirds that are known for their complex learned song that consists of several 'song phrase types' which are repeated two or more times before the next phrase type is sung (Eens 1997). The grammar stimuli were constructed from two of these natural phrase types: 'rattles' and 'warbles'.

Gentner *et al*. (2006) trained starlings in a Go/No-Go experiment, a different paradigm than the habituation/dishabituation experiment that was used to test discriminatory abilities in tamarins. In this Go/No-Go experiment, the bird has to respond to one type of stimuli, but withhold responses to the other. Contrary to the tamarin experiment by Hauser and Fitch (2004) in which recognizing each grammar was tested separately by presenting violations (the other grammar) in a habituation/dishabituation experiment, in this Go/No-Go experiment, the discrimination between the two grammars was tested directly. The authors reported that starlings, unlike the cotton-top tamarins, were able to "accurately recognize a recursive, self-embedding, context-free grammar and to reliably exclude agrammatical patterns and classify new patterns defined by the grammar". This claim entails that the capacity to detect recursion in auditory sequences would not be uniquely human. In support of the claim, the study included a range of controls for alternative (simpler, but still abstract) rules that the birds could have used to distinguish the two stimuli sets.

Like the tamarin experiment, the starling experiment by Gentner *et al.* (2006) received a (similar) variety of comments that were brought up before in response to HCF (2002a) and Fitch and Hauser (2004). These mainly concerned the actual recursiveness of the test stimuli and the way they were presented; the level of abstract rule learning that the starlings showed; the relatively high number of trials the starlings needed to learn the task and the correctness of the implications the authors mention for the unique characteristics in human language (Corballis 2007a and b; De Vries *et al.* 2008; Hochmann *et al.* 2008; Liberman 2006; Premack 2007; Marcus 2006).

But while the discussion on the experiment in terms of whether it is an appropriate test of the presence of recursion lingers on, a more basic question about what the starlings have actually learned has received little attention (but see Corballis 2007a; Marcus 2006): did they learn an abstract rule allowing them to generalize from familiar to novel stimuli? Gentner *et al.* (2006) showed that starlings generalized from familiar 'rattle' and 'warble' structures to unfamiliar ones, but not that they generalized to *other* phrase types as well. Therefore, the birds could also have based their discrimination on a phonetic categorization, restricted to rattle and warble phrase types. I.e. they might classify all 'rattle-warble-rattle-warble' sequences as one type, different from 'rattle-rattle-warble-warble' sequences, based on the overall acoustic similarity alone (and with the appropriate similarity metric, this would generalize to larger *n* without a need for a syntactic analysis, (Suhara and Sakurai 2007)). So the question whether the birds have the abstract computational abilities that enable them to discriminate between the two stimuli sets strictly based on order information is still open.

To test this, the birds should be presented with a new set of song stimuli, consisting of new phrase types (for instance structures constructed of C's and D's). In this case, the birds cannot use phonetic generalization, but have to attend to the sequence of phrase types, requiring some level of abstract computational skill. In the current paper, we present such an experiment with another songbird species, the zebra finch (*Taeniopygia guttata*). Using a similar setup as the Gentner *et al.* study (2006) – including, importantly, the use of a probe methodology to test for a range of alternative hypotheses – we extend their results by explicitly testing for generalization and by a more in-depth statistical analysis of the probes.

The zebra finch is widely used as a model species in neuro- and behavioral sciences and knowledge about the perceptual abilities of this species would be greatly beneficial to these research areas. An additional reason for choosing the zebra finch is the suggestion that the complex perceptual ability in starlings could be related to the relatively complex structure of their natural songs (Yip 2006). The song of the zebra finch is relatively simple, consisting of a limited number of elements and a relatively stereotyped sequence (e.g. Price 1979; Scharff and Nottebohm 1991; Zann 1993b); Sturdy *et al.*1999a; Holveck *et al*. 2008). Nevertheless, zebra finches are able to discriminate between songs with subtle differences such as the number of repeated elements and the relative position of a certain element in a song (Verzijden *et al*. 2007). So our experiment also tests whether birds with much simpler songs are able to detect more abstract patterns in vocal structures.

The results from this experiment show that zebra finches, like starlings, can discriminate between sets of two syntactically different song stimuli and can generalize this discrimination to unfamiliar exemplars of the same stimulus types. In addition, when confronted with novel stimulus types arranged identically to the earlier ones, one out of eight birds was able to correctly classify the stimuli, an ability that so far has not been shown in any other songbird. However, additional 'probe tests' to examine the rules that the birds used to discriminate between syntactically different sets, suggest that all birds used simpler rules to discriminate between the sets with and without 'recursion'.

RESULTS

Transfer from 1 song to 5 songs of each structure

During the training phase, the birds learned to discriminate between a single ABAB and one AABB stimulus (see fig. 3.1a and b). As the two structures consisted of identical elements, the only way to discriminate between the two is by learning about the order of the individual elements within the structures, an ability demonstrated in zebra finches before (Verzijden *et al*. 2007).

The simplest mechanism to do this would be to learn the sound characteristics of each structure i.e. by rote memorization. We tested this by presenting additional stimuli (2x4) of the same structures but constructed of different element exemplars. If the birds based their discrimination on the individual sound characteristics of the two stimuli, they would perceive this as a new task and show a considerable drop in DR after transfer to around 0.50 and a d' of around zero, meaning no discrimination (see (MacMillan and Creelman 2005) and (Gentner *et al*. 2006) for method of calculating the d' and its confidence interval). Alternatively, if the birds learned a more general rule either related to the structure of the sounds, or their phonetic structure, they should be able to generalize to the additional stimuli and show a DR higher than 0.50 (and a d'

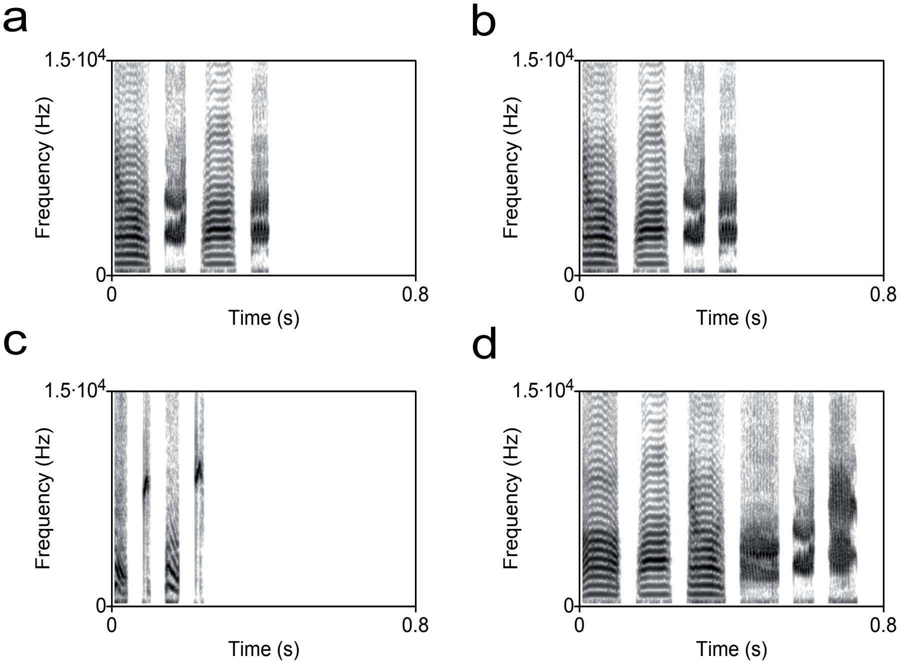

Fig. 3.1. Spectrograms of constructed song stimuli. **a**) stacks and trills in ABAB order. **b**) stacks and trills in AABB order. **c**) slides and highs in ABAB order. **d**) stacks and trills in AAABBB order. Note that the different panels show different exemplars of the different elements used. In particular fig. d illustrates within and between element type variation in the stimulus sets.

higher than zero). Fig. 3.2b shows the average discrimination ratios per 100 trials before and after transfer to 2x 5 ABAB's and AABB's. Directly after transfer, there is an initial drop of the DR to 0.56, which is slightly higher than random performance, but the birds learned to discriminate faster than during the previous task. This can be concluded from the d' measures: for the first two 100 trial blocks six out of eight birds discriminated significantly between the two sets and seven out of eight were able to do so during blocks 3-5 after transfer (see table 3.1). Hence, we can conclude that most birds were able to generalize to the additional stimuli.

Transfer from 5 songs of each stimulus type to 5 novel songs of each type

With additional training, all birds reached criterion performance. However, the question remains whether the birds learned an abstract computational rule to discriminate between these ABAB songs and AABB songs or whether there is a lower level explanation (i.e. learning the ten songs by rote memorization or by phonetic generalization).

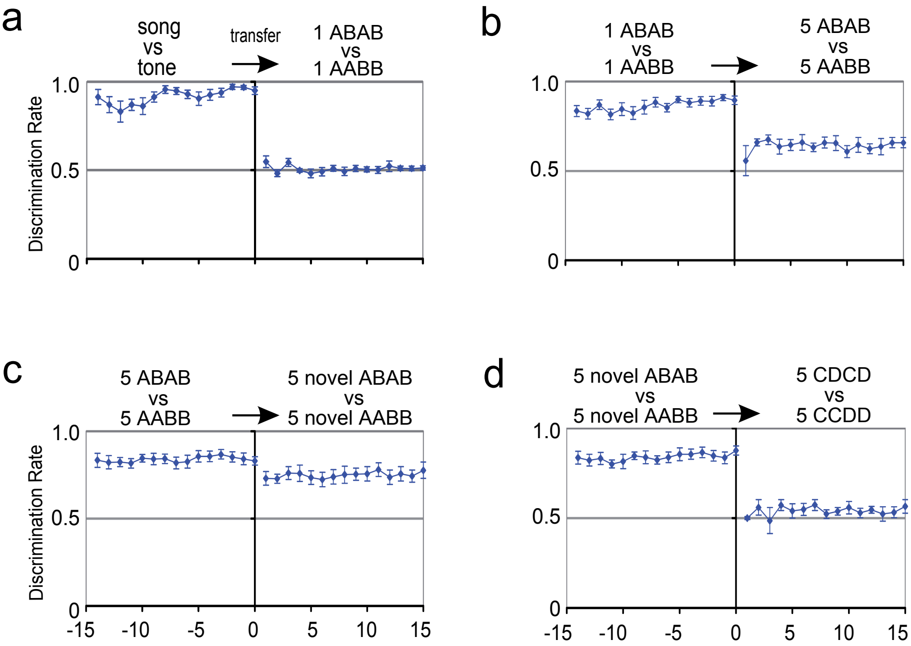

Fig. 3.2. Average Discrimination Ratio's per blocks of 100 trials before and after transfer to new stimuli (± s.e.m.).

Table 3.1. Average d''s after each transfer (± s.e.m.). Min L bound CI: lowest lower bound of the confidence interval (of individual birds). Max L bound CI: highest lower bound of the confidence interval (of individual birds).

transfer		**blocks 1 and 2**				**blocks 3 to 5**			
from	**to**	**d'**	**s.e.m.**	**min L bound CI**	**max L bound CI**	**d'**	**s.e.m.**	**min L bound CI**	**max L bound CI**
song/tone	1 ABAB/ AABB	0.04	0.09	-0.54	0.21	0.07	0.06	-0.53	-0.01
1 ABAB/AABB	5 ABAB/ AABB	0.67	0.13	-0.33	0.83	0.68	0.16	-0.25	1.08
5 ABAB/AABB	2 x 5 novel	1.10	0.16	-0.09	1.29	1.31	0.17	0.32	1.67
2 x 5 novel	2 x 5 new element types	0.09	0.07	-0.47	-0.03	0.25	0.10	-0.47	0.40

To test whether the zebra finches are able to generalize independent of the characteristics of the individual elements in the stimuli, a different set of stimuli of the same element categories was introduced. From stimuli constructed of $a_{1\text{-}6}$ and $b_{1\text{-}6}$, each bird was transferred abruptly to stimuli constructed from $a_{6\text{-}10}$ and $b_{6\text{-}10}$ when it reached criterion performance (see table SI 3.1 for stimulus details). See fig. 3.2c for average discrimination ratio's per 100 trials before and after transfer to 2x 5 novels songs of each structure. After transfer, the average DR remained above 0.50. The slight drop indicates that the birds do notice a difference between the first and second sets, but they treated the novel stimuli similar to the previous ones. Seven out of eight birds generalized to the new stimuli within the first 2 blocks and all birds did so within blocks 3-5 (see table 3.1). This outcome suggests that they used a general rule instead of rote memorization of all individual songs to base discrimination on.

It is here that the question arises what exactly the birds use to generalize: an abstract rule (as suggested by Gentner *et al*. 2006), or a lower level process, such as phonetic generalization. The use of an abstract computational rule would predict a transfer of the discrimination to songs of *any* element type, as long as they occur in a same order. The question thus is whether the birds can generalize *independent* of element *type*.

Transfer from 5 songs of each stimulus type to 5 songs with new element types (A's and B's to C's and D's)

To test whether the zebra finches are able to generalize to new songs *independent* of element type, each bird was transferred abruptly to C's and D's, i.e. other types of elements instead of A's and B's, when it reached criterion performance (see fig. 3.1c). The average DR's before and after transfer are shown in fig 3.2d. The average DR after transfer stayed just above 0.50, but all d''s for the initial two blocks after transfer were not significant. For blocks 3-5 however, three of the eight birds showed significant discrimination (see table 3.1). However, two of these three birds dropped to chance performance again (DR of 0.50) shortly after this interval. But the third bird, m574, remained on a high discrimination level (see fig. 3.3). Only after training did the seven other birds learn to discriminate between the new stimuli sets, indicating that they perceived this as a new task.

So we conclude that seven out of eight birds were unable to generalize the discrimination of the stimuli to novel ones using structure cues only. However, there was one zebra finch that showed a clearly different response pattern and was likely to be able to generalize *independent* of element types and therefore used a more abstract general rule.

Testing for the use of other, simpler rules to discriminate between ABABs and AABBs

One bird (m574) showed that he was capable of generalizing across element types and so apparently used a more abstract rule for distinguishing between the stimuli sets than the other birds. However, the rule it used might not have been 'recursive', but could have been a more simple, lower level strategy. For instance, ABAB and AABB differ in their first two elements and therefore can be discriminated

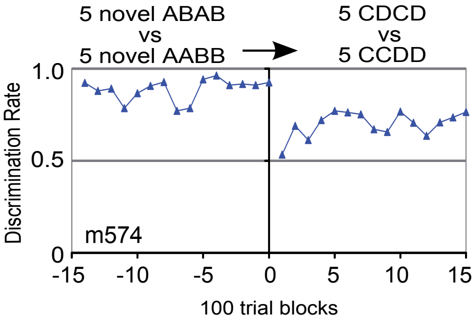

Fig. 3.3. Discrimination Ratio's per blocks of 100 trials of bird m 574 before and after transfer to song stimuli of new element types.

based on this difference instead of all four elements ('primacy rule'). Similarly, discrimination might be based on the last two elements only ('recency rule').

These lower level rules were tested in a probe testing phase during which several probes, consisting of 'c' and 'd' elements, were presented (see Table SI 3.1), similar in design to the probes used in Gentner *et al.* (2006). In this testing phase, four birds were exposed to probe songs. Probe songs were not reinforced in order to avoid additional learning and presented in only 20% of all stimulus presentations to prevent extinction of the pecking behavior. The other 80% of stimulus presentations were the reinforced stimulus songs to which the birds were exposed during the previous phase (table 3.2 provides individual responses to these probes – see fig. 3.1d, an example).

To test whether the birds showed a differential response to the probes, we tested for each bird individually whether the responses to the probes differed, which it did (binomial test in R, y~probetype, family=quasibinomial). All deletion p-values for probe type were significant (largest p-value was <0.05) indicating that all four birds did not respond in a random manner to the probe songs.

However, the pattern of responses to probes did not fit either of the grammars used to define the training stimuli. Like Gentner *et al.* (2006), we considered the alternative hypotheses that for distinguishing the training stimuli the birds used a strategy based only on the first transition in each sequence (AA vs. AB, 'primacy'), the last transition (BB vs. AB, 'recency') or any transition (the presence vs. absence of AA, BB or BA, 'bigram'). Gentner *et al.* (2006) try to exclude these alternative strategies by showing that the birds are significantly better at distinguishing between the two sets of training stimuli than between a contrasting pair of probe stimuli. That is, the d' between ABAB and AABB is significantly larger than between BAAB and ABBA, which Gentner *et al.* (2006) take as excluding the bigram hypotheses.

Table 3.2. Average response to the two consecutive blocks of 30 probes of each probe type.

		probe type								
bird ID	S+	ABAB	AABB	AAAB	AAAA	BBBB	BAAB	ABBA	AAABBB	AAAABBBB
570	AABB	1.5	28.0	22.5	22.5	0.0	8.5	1.5	17.5	14.0
574	AABB	1.0	24.5	8.0	8.0	23.0	9.5	1.5	20.0	21.0
583	ABAB	22.0	0.0	0.0	0.0	25.0	2.5	26.0	0.0	0.5
602	ABAB	25.0	3.0	4.5	2.0	22.0	1.0	26.5	1.0	1.0

However, given that the d' is significantly smaller for all pairs of probe stimuli, including the 'context-free' probes AAABBB and AAAABBBB, that is not the relevant test. We performed a maximum likelihood analysis instead, evaluating for every individual bird which of the considered alternative hypotheses best explains the observed data. From such an analysis (as shown in table 3.2) we deduced that m574, the bird that was able to generalize to new element types, did so without using the 'context-free' strategy. If he had acquired this strategy, he should not respond to the following probes, AAAB, AAAA, BBBB, BAAB and ABBA. The results show that he did in fact respond to for instance, BBBB. His pattern of response best fits a BB-recency strategy (responding selectively to sequences ending in BB). Two other birds most likely used 'bigram AA' to distinguish between the sets; one bird used 'primacy AA'.

DISCUSSION

This paper addressed the question whether songbirds are able to discriminate between vocal structures with and without a 'center-embedded recursive' structure. The results of our experiment show that all zebra finches learned to discriminate between two stimulus sets that solely differed in the order of the elements. This shows that a songbird species lacking complex and variable songs *can* be highly sensitive to song structure, corroborating results in a different context by Verzijden *et al*. (2007).

We show that zebra finches are able to generalize from training exemplars to new song stimuli consisting of new elements of the same element types. A number of birds showed a slightly delayed generalization response after certain transfers to new stimuli. The magnitude of their discrimination (described by the DR and d') was reduced directly after transfer (blocks 1-2), which would not be expected for generalization. However, their recovery during blocks 3-5 was faster than would be expected if they had perceived this as a new task (i.e., compared to for instance, the response behavior after transfer to the first ABAB and AABB).

Until this stage, the experiment ran parallel to Gentner *et al*. (2006).

However, our main objective was to test whether songbirds are able to generalize independently of phonetic characteristics of the element types that are used in the stimulus songs. The results of our additional transfer stage showed that only one bird was able to discriminate the stimuli independent of element type. This zebra finch was therefore able to use more abstract skills than the others. However, additional testing showed that, just as other zebra finches, this bird did not use a 'recursive' strategy, but, a simpler strategy (although still abstracting over phonetic detail). From an efficiency point of view, this makes sense as it would be inefficient to use relatively complex rules, when simpler rules suffice. We conclude that the current experimental paradigm, although yielding interesting results, has not answered the questions whether zebra finches have the abilities to detect recursion.

However, this conclusion also applies to previous studies on this issue. For the starling experiment of Gentner *et al*. (2006) for instance, it is still not clear whether the data allow the rejection of the *primacy rule*, for two reasons.

First, the mean d' between AAAA and ABBA was significantly lower than between AABB and ABAB, but it was still significantly above 0 (0.99 ± 0.40 s.e.m.). Consequently, it cannot be ruled out that the starlings (at least partly) used a primacy rule instead of a 'recursion' rule.

Secondly, our zebra finches used different individual strategies (see table 3.2) to discriminate between the stimulus songs. Gentner *et al*. (2006) reported the *mean* d' ± s.e.m. instead of the individual d''s. Therefore, if starlings also show individually different strategies, every other comparison than between AABB and ABAB is likely to produce a less clear result, as all other comparisons are a mixture of one or more birds that did or did not use the tested strategy. This would mean that contrary to the authors' conclusions, the evidence whether starlings are able to detect recursion is inconclusive.

Interestingly, the same uncertainty remains about human abilities in artificial language learning tasks. The findings in Fitch and Hauser (2004) lacked the appropriate controls, but later replications claim that the AABB/ABAB task, which Fitch and Hauser (2004) designed to obtain evidence for recursion, can be solved by humans using a simpler strategy instead (Perruchet and Rey 2005; De Vries *et al*. 2008) or by a conscious counting strategy that seems unrelated to language (Hochmann *et al*. 2008). At present, there is thus no convincing demonstration of the use of recursive rules in artificial language learning in any species. It remains a challenge to design experiments on artificial rule learning and its underlying mechanisms that unambiguously exclude simpler explanations for discrimination between training structures, a problem that also faces experiments in humans (e.g. Seidenberg *et al*. 2002). Combined with the fact that it is far from clear that recursion actually is the only unique aspect of human language (see for instance Christiansen and Kirby, 2003 and Pinker and Jackendoff, 2005), or even a particularly relevant feature (Verhagen 2009), it might be time to switch the attention to the pattern recognition abilities of zebra finches and other species, which are remarkable in their own right, quite independently from the question of the origin of recursion in human language. With this study, we have uncovered some details of those abilities in zebra finches, extended the experimental paradigm to address such questions and hopefully contributed to putting the claim for or against the human uniqueness of recursion in the right perspective.

MATERIALS AND METHODS

Subjects and Housing

Eight zebra finches from our breeding colony (six males and two females; age range: 137-363 days at the start of the experiment) were individually trained and tested in this experiment. They had no previous experience with similar experiments.

Before the experiment, the birds were housed in the breeding colony in same sex groups of two to six individuals and on a 13.5 L:10.5 D schedule at 20-22 °C. Drinking water, cuttlebone and a commercial tropical seed mixture enriched with minerals was available *ad libitum*. Twice a week, the birds received some egg food and seedlings from the seed mixture.

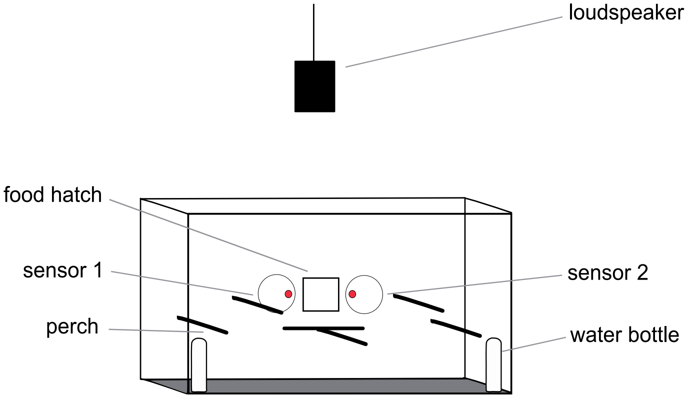

Fig. 3.4. Learning the Go/No-Go procedure in a Skinner box. A peck on the left sensor, when its LED was on, initiated a playback of a positive 'Go' stimulus song (S^+, the natural zebra finch song motif). After this song was finished, the LED in the right sensor was switched on and pecking on it opened the food hatch for 10 s (positive reinforcement). When the bird pecked at the right sensor within 6 sec response interval for at least 75%, the negative 'No-Go' stimulus (S^-, the tone), was introduced in 60% of the cases the bird pecked the left sensor. Subsequently, the bird had to learn not to peck at the right sensor after hearing an S^- stimulus, or the lights would go out for 15 sec (negative reinforcement).

Apparatus

During the experiment, subjects were individually housed in an operant conditioning chamber also known as a Skinner box (70 (l) x 30(d) x 45(h) cm), constructed of wire mesh front and side walls, and a ply wood back wall (see fig. 3.4). The cage was placed in a sound attenuated chamber. Water and cuttlebone were available *ad libitum*. A fluorescent tube (Lumilux De Luxe Daylight, Osram) was placed on top of the cage, with the same light/dark schedule as the breeding colony (except during punishment reinforcement, see Go/No-Go procedure). The back wall contained two red pecking sensors with red LED lights and a food hatch. Stimuli were played through a loudspeaker (Vifa MG10SD09-08) located 1 m above the operant conditioning chamber and calibrated to a (peak) output of approximately 70 dB (SPL meter, RION NL 15, RION) at the food hatch. The fluorescent tube, the two sensors, the food hatch and loudspeaker were connected to a custom-made operant conditioning chamber controller that also registered the sensor pecks. To monitor their condition, the birds were weighed before and after the experiment and their food intake was measured daily. The study was conducted according to Association for the Study of Animal Behaviour guidelines on animal experimentation as well as to the Dutch law on animal experimentation. The Leiden Committee for animal experimentation (DEC) approved the experiment under number 06150.

Song stimuli construction

We constructed stimulus songs, each containing four units, as in the earlier studies (Fitch and Hauser 2004; Gentner *et al.* 2006). In our case, these units were elements obtained from natural zebra finch song from our zebra finch song database (consisting of undirected song, for recording specifications see (Holveck *et al.*, 2008).

Four element types occurring in natural zebra finch song (‘flats’, ‘slides’, ‘highs’ and ‘trills’ (see fig. 3.1) were selected that were easily distinguishable by visual inspection. The first three have previously been recognized as element types by several authors (e.g. Price 1979; Scharff and Nottebohm 1991; Zann 1993b; Sturdy *et al.* 1999a). The fourth, the ‘trill’, characterized by rapid and repeated frequency modulation and, is less well known but occurs in songs from our lab (Holveck *et al.* 2008) and has been previously described as a ‘click’ (Williams and Staples 1992) and as a ‘buzz’ (Leadbeater *et al.* 2005).

An element library of 40 (10 x 4) elements was constructed by selecting elements from songs in our zebra finch song lab database. All elements (as well as the shaping stimuli) were ramped (3 ms) and rms (0.1) equalized in PRAAT (version 4.5.08, www.praat.org).

Subsequently, 40 stimulus songs were constructed, each consisting of four different elements, with 40 ms as an inter element pause. Each four-element song consisted of two element types, A's and B's and were ordered in either the ABAB structure or the AABB structure. In a later phase we used elements of another type (C's and D's - see section 'transfer to new element types'). Songs always consisted of non-identical elements within element types. So $a_1b_1a_2b_2$ was a member of the ABAB type set and $a_1a_2b_1b_2$ for its equivalent in the AABB type set. (For more information on probe stimuli construction, see SI.)

In order to pre-train the birds for the experiment, two other stimuli were constructed which were similarly ramped and equalized as the elements from the element database: a natural zebra finch song motif, 0.58 sec in duration, randomly chosen from our zebra finch song database and a 2 kHz tone of identical duration as the S^+, also constructed in PRAAT.

To control for order effects, the element types of the stimuli songs that the experiment was started with (A's and B's) was counterbalanced between birds. So the other half of the birds started with C's and D's and were in this phase transferred to A's and B's.

Go/No-Go procedure

The birds were trained in a Go/No-Go procedure with a food reward to test their ability to discriminate between stimuli of the ABAB and AABB structures.

A peck on the left sensor, when its LED was on, initiated a playback of a positive 'Go' stimulus song (S^+, the natural zebra finch song motif). After this song was finished, the LED in the right sensor was switched on and pecking on it opened the food hatch for 10 s (positive reinforcement). When the bird pecked at the right sensor within 6 sec response interval for at least 75%, the negative 'No-Go' stimulus (S^-, the tone), was introduced in 60% of the cases the bird pecked the left sensor. Subsequently, the bird had to learn not to peck at the right sensor after hearing an S^- stimulus, or the lights would go out for 15 sec (negative reinforcement).

After the bird reached criterion performance (>75% response after S^+ and <25% response after S^- for at least two full consecutive days) and a phase where the ratio of S^+/S^- was set to 50/50 (in random order), the training with the two stimuli sets began. The type of stimulus, either ABAB or AABB, was mapped on the S^+ and S^-, and was counterbalanced between birds. So half of the birds received the ABAB songs as the S^+ stimuli and the AABB songs as the S^- stimuli.

Statistics

Discrimination by each zebra finch was visualized by plotting the DR (discrimination ratio, calculated as the percentage response to S^+ divided by the sum of the percentage response to S^+ and the percentage response to S^-), but was measured with d' (both per 100 trials), which is independent of response bias (MacMillan and Creelman 2005). A Confidence Interval for d' with a lower bound above 0 was considered as significant discrimination between two stimuli sets. Two intervals after transfer to a new set of stimuli were tested: the first two blocks and blocks 3 to 5. See Gentner *et al*. (2006) and MacMillan and Creelman (2005) for the method of calculating the d' and its confidence interval. Some birds showed a continued response bias during one or more phases. If this occurred in combination with a very low S^-, and hence a high DR we decided to transfer the particular bird to the next phase when their DR was higher than 0.75 for at least two full consecutive days (alternative learning criterion).

For the statistics on the data from the probe testing, see SI.

Training phase: transfer from song and tone to 1 ABAB and 1 AABB song

After the pre-training with a song and a tone and the birds reached criterion performance, the stimuli were abruptly changed (transferred) to one exemplar of ABAB and one of AABB. Just before transfer, the birds (n=8) had an average d' of 3.49 ± 0.26 (s.e.m.) and a range for the lower bound of the 95% confidence interval (CI range) of 1.95-3.45 calculated for the last two blocks of 100 trials each.

It can be seen in the graph (fig. 3.2a, DR around 0.50) and from the d' value and its CI range in table 1 (d' 0.04 ± 0.09, lower bounds of the individual CI's between -0.54 and - 0.21) that this is indeed the case for the first two blocks of 100 trials after transfer (d' is near zero, which means no discrimination). The birds seem to start from scratch again, i.e. there was no obvious transfer of the Go/No-Go paradigm to novel stimuli, as indicated by the finding that none of the birds was significantly discriminating between the sets during blocks 3-5 after transfer. (One bird did show significant discrimination in these first two 100 trial blocks, but thereafter his d' dropped below 0 and was no longer significant during blocks 3-5.)

However, after training, all birds discriminated the AABB and ABAB stimuli on or above criterion level.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY INFORMATION

Probe stimuli construction

In order to avoid novelty effects in the responses of the birds due to the presence of new element type exemplars in the probe stimuli, the probes were constructed from the same set of exemplars as the birds heard in the previous phase. However, the combination of element type exemplars was new. For instance, whereas in the previous phase, the bird heard $a_1b_1a_2b_2$ to $a_4b_4a_5b_5$, we included stimuli such as $a_4a_1a_3b_5$ in 'shuffle' play.

Maximum Likelihoods for individual rules used by the zebra finches

In order to calculate the Likelihoods, we did two analyses: one based on the fraction Go in response to the training stimuli (the reinforced ones) and one based on the fraction Go in response to the probe versions of the training stimuli. Both rendered similar outcomes. For each bird, we calculated the chance of responding to the probes according to a specific rule, by calculating the binomial chance given the number of responses to a certain probe. These chances were than log transformed and added for all probes. The rule that fitted the data best (highest LL score) was then taken as the most likely rule the bird used to discriminate between the stimuli sets (ABAB and AABB).

Table SI 3.1. Stimulus schedule. (taken from ten Cate, van Heijningen and Zuidema 2010) **a**) A, B, C, and D indicate element types; a, b, c, and d indicate element exemplars. Training began with a single ABAB and AABB stimulus ($a_1b_1a_2b_2$ vs. $a_1a_2b_1b_2$), subsequently extended to all five AABB and ABAB stimuli. ABAB stimuli were composed from six different bigrams, with four present twice, in different positions. Each ABAB stimulus had a matching AABB stimulus constructed from the same elements. Hence, no stimulus could be recognized by learning the constituting elements only, forcing the birds to pay attention to element order. **b**) Subsequent probe session in stimulus schedule. Our probe testing occurred after transfer to stimuli with 'c' and 'd' elements and after reaching discrimination. These elements were used subsequently, in novel combinations, in the probe testing phase. *Probes with 'ABAB' and 'AABB' structure. These, as well as almost all others, shared no bigrams with the training stimuli for this phase.

a

Training							
ABAB				**AABB**			
a1	b1	a2	b2	a1	a2	b1	b2
a2	b2	a3	b3	a2	a3	b2	b3
a3	b3	a4	b4	a3	a4	b3	b4
a4	b4	a5	b5	a4	a5	b4	b5
a5	b5	a6	b6	a5	a6	b5	b6
Transfer to five novel songs of each type							
ABAB				**AABB**			
a6	b6	a7	b7	a6	a7	b6	b7
a7	b7	a8	b8	a7	a8	b7	b8
a8	b8	a9	b9	a8	a9	b8	b9
a9	b9	a10	b10	a9	a10	b9	b10
a10	b10	a1	b1	a10	a1	b10	b1
Transfer to songs of new element types, but same structure							
CDCD				**CCDD**			
c1	d1	c2	d2	c1	c2	d1	d2
c2	d2	c3	d3	c2	c3	d2	d3
c3	d3	c4	d4	c3	c4	d3	d4
c4	d4	c5	d5	c4	c5	d4	d5
c5	d5	c6	d6	c5	c6	d5	d6

b

Subsequent probe session	
structure	**elements**
CDCD*	c1 d3 c5 d2
CDCD*	c2 d5 c4 d3
CCDD*	c1 c5 d3 d2
CCDD*	c2 c4 d5 d3
CCCD	c4 c1 c3 d5
CCCD	c5 c2 c1 d4
CCCC	c1 c4 c5 c2
CCCC	c2 c5 c4 c3
DDDD	d1 d3 d5 d2
DDDD	d2 d5 d4 d3
DCCD	d3 c5 c2 d5
DCCD	d4 c3 c5 d1
CDDC	c3 d4 d2 c1
CDDC	c4 d1 d3 c2
CCCDDD	c4 c2 c1 d4 d1 d3
CCCDDD	c5 c3 c1 d2 d5 d4
CCCCDDDD	c3 c1 c4 c2 d4 d3 d1 d5
CCCCDDDD	c4 c2 c5 c3 d1 d5 d4 d2

Chapter 4

Rule learning by zebra finches in an artificial language learning task: which rule?

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ABSTRACT

A hallmark of human language is the use of syntactic rules. Animals do not use complex syntactical rules in their natural vocalizations, but several studies indicate that they can detect differences in the syntactical structure of acoustic stimuli. Which rules they can learn however, and how exactly they solve various experimental tasks is often not clear. Using an Artificial Grammar Learning (AGL) paradigm, zebra finches were tested in a Go/No-Go experiment for their ability to distinguish structurally different three-element song sequences. Eight out of ten zebra finches learned to distinguish ABA and BAB from ABB, AAB, BBA and ABB sequences. Tests with unfamiliar probe sounds showed that only one bird generalized the discrimination to a new element type. Other probes suggested that discrimination between the stimulus sets resulted from attending to the presence or absence of repeated elements. We continued the training by adding four-element songs following a ‘first and last identical vs. different’ rule that could not be solved by attending to repetitions. Only two out of five birds learned the overall discrimination. Testing with novel probes demonstrated that discrimination was not based on using the ‘first and last identical’ rule, but by attending to the presence or absence of the individual training stimuli. The two birds differed in the strategies used. Our results thus demonstrate only a limited degree of rule learning, but highlight the need for extensive and critical probe testing to examine the rules that animals (and humans) follow in AGL tasks. They also underline that rule learning strategies may differ between individuals.

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Chapter 4

INTRODUCTION

A key requirement for language learning is the ability to detect structure and the rules underlying structure when exposed to a stream of speech sounds. Major advances in understanding this ability originate from experiments using Artificial Grammar Learning (AGL), in which subjects are exposed to artificially constructed strings of speech sounds (e.g. syllables) devoid of any meaning, but structured according to a particular rule. Whether subjects detect the underlying rule when exposed to the sound stream or use another can be traced by examining the responses to novel sounds. The AGL paradigm has proven extremely powerful to analyze the rule learning abilities of human infants. Some of the major findings are the discoveries that eight month old infants are able to track transitional probabilities in a continuous stream of syllables and use this to distinguish 'words' from 'non-words' (Saffran *et al*. 1996), and the discovery that seven month old infants can detect an XYY or XYX pattern in a sequence of syllables and generalize this to sequences consisting of new syllables (Marcus *et al*. 1999).

Another major conclusion obtained by AGL studies is that mechanisms for language learning need not be specific to language learning alone, but might involve more domain general ones (reviewed by Gomez and Gerken, 2000 and Folia *et al*. 2010) and show up with non-linguistic stimuli like musical tones and other non-linguistic sounds (Endress, 2010; Gebhart *et al*. 2009; Saffran *et al*. 1999, but see Creel *et al*. 2004; and pictures, Saffran *et al*. 2007). Findings like these raise the question which aspects of language learning can be explained by general perceptual and computational mechanisms and which by mechanisms that are specific for language acquisition (Newport and Aslin 2004; Saffran *et al*. 2008; Endress *et al*. 2009b). In addition to rule learning and statistical learning mechanisms contributing to language learning, it has been demonstrated that early language learning can be guided by POMPs, Perceptual Or Memory Primitives, like sensitivity to edges of strings or repeated units (Endress *et al*. 2005; Endress *et al*. 2007; Endress *et al*. 2009b). These POMPS are also suggested to provide a basis for the evolution of linguistic learning mechanisms.

While examining rule learning of humans using non-linguistic stimuli is one way to address the questions of domain specificity, another important one is to explore their presence in non-human animals (Hauser *et al*. 2002a; Endress *et al*. 2009b): how do animals cope with various rule learning tasks?

Regarding rule learning, an increasing number of species has been tested and found able to detect regularities in acoustic input; primates like chimpanzees (Endress *et al*. 2010), rhesus monkeys (Hauser and Glynn 2009), tamarins (Hauser *et al*. 2001; Newport *et al*. 2004), but also more distantly related species such as rats (Toro and Trobalon 2005; Murphy *et al*. 2008), starlings (Gentner *et al*. 2006), zebra finches (van Heijningen *et al*. 2009) and Bengalese finches (Abe and Watanabe 2011, but see Beckers *et al*. 2012).

In the current study we address the ability of birds for rule learning of the form XYX (X and Y denoting arbitrary sound item categories), where the first element is identical to the last one in the sequence and differentiating it from XXY and XYY. Marcus *et al*. (1999) showed that seven month old infants can distinguish XYX from XYY speech stimuli. Regarding the abilities of non-human animals to detect such a structure, the results are ambiguous. The first study to address this, using a habituation paradigm in tamarin monkeys (Hauser *et al*. 2002b) suggested that tamarins could resolve the task, but this paper was withdrawn recently (Hauser *et al*. 2010). No evidence for discriminating XXY from XYY in human speech stimuli by rule learning was obtained in a study in rats using a habituation paradigm (Toro and Trobalon 2005). Still, Hauser and Glynn (2009) showed that rhesus monkeys could discriminate XYY from XXY when X and Y were rhesus monkey calls.

Recently, using an operant paradigm, Murphy *et al*. (2008) showed that rats could distinguish an XYX (i.e. ABA, BAB – A being a bright light, B being dim light), from an XYY (i.e. ABB, BAA) and a XXY (i.e. AAB and BBA) configuration (see also Fountain and Rowan 1995), with one of these configurations signaling food. A second experiment in which A and B were tones tested whether the rats could transfer the discrimination from one set of tones to a novel one. Perhaps surprisingly, the rats could do so. Fascinating as this finding might be, it remains, however, unresolved how the rats made the distinction between the sequences (see also Corballis 2009). The authors suggest that the rats learned some overall rule, rather than the individual strings, but neither presence nor nature of this rule can be deduced from this result. The rats might, for instance, have learned to respond only to a structure without immediate repetition, without noticing the similarity of first and last item. Also for the earlier experiments on animals, e.g. testing for distinguishing XXY from XYY (Hauser *et al*. 2002b, retracted; Hauser and Glynn 2009; Toro and Trobalon 2005) the emphasis was on showing that the animals could transfer from a training set of specific X's and Y's to a novel set, rather than exploring the particular rule used by the animals. Thus, studies examining these rules in any detail are still lacking.

Arguably the best, but surprisingly hardly used model species for examining rule learning abilities in animals are songbirds. In contrast to most mammal species, songbirds have complex, learned vocalizations, and birdsong researchers have discovered remarkable cognitive, neural and genetic parallels between the processes involved in the production, perception and development of language and birdsong (Berwick *et al*. 2011; Bolhuis *et al*. 2010; Doupe and Kuhl

1999). The first study to address vocal rule learning in a bird species was one in which starlings were examined for their ability to detect a ‘recursive’ structure. Gentner *et al*. (2006) examined the ability of starlings to discriminate the recursive A^nB^n structure from $(AB)^n$ sequences in a so-called Go/No-Go design using probe tests to explore the rule used by the starlings. In our studies on acoustic stimulus generalization (Verzijden *et al*. 2007) and rule learning (van Heijningen *et al*. 2009) we use zebra finches. Zebra finches, like starlings, can also learn to discriminate AABB from ABAB sequences. However, further tests suggest that this ability seems to result from using more simple mechanisms such as acoustic generalizations or attending to the presence of repeated elements), also providing an alternative explanation for the results obtained with starlings (Corballis 2007a; ten Cate et al. 2010). Nevertheless, a recent study on Bengalese finches (Abe and Watanabe 2011) suggests more complex rule learning in birds, demonstrating the suitability of birds for addressing question of rule learning in the context of vocal stimuli (but see Beckers *et al*. 2012).

In the current study we trained zebra finches to distinguish ABA and BAB from AAB, ABB, BAA and BBA (i.e, similar to the stimulus configurations used by Murphy *et al*. 2008). Next, we used probe stimuli to explore the rules by which the birds might make the distinction. In a subsequent experiment, we added an additional set of training stimuli in order to make the birds switch to a different rule for discriminating between the stimulus sets, and again use probe tests to explore the outcome.

GENERAL METHODS/RESULTS

Subjects and Housing

Ten adult zebra finches (at least 120 days old, five females and five males) from our own breeding colony were trained and tested in individual operant conditioning chambers using a Go/No-Go procedure. The birds were naïve to the setup and training. In the breeding colony, adult birds are housed in same-sex aviaries on a 13.5 L: 10.5 D schedule at 20-22 ºC. Cuttlebone, drinking water and commercial tropical seed mix (Tijssen, Hazerswoude) enriched with minerals were available *ad libitum*. The birds received a limited amount of egg food and fresh seedlings twice a week.

Apparatus

The birds were individually housed in operant conditioning chambers also known as Skinner boxes [70 (l) x 30 (d) x 45 (h) cm] made of wire mesh and a ply wooden back wall. The floor was covered with sand and grit. A fluorescent tube on top of the chamber emitted daylight spectrum light (Lumilux DeLuxe Daylight, Osram) on an identical light/dark schedule as in the breeding colony room except for negative reinforcement periods (see Go/No-Go procedure) in which the light was temporarily switched off. The back wall contained a food hatch and two red pecking keys, each containing a red LED (see Go/No-Go procedure). The pecking keys and food hatch can be reached from wooden perches, with four additional perches to enable normal hopping behaviour. A small bird mirror was placed on a side wall as cage enrichment.

Sound stimuli were played via a loudspeaker (Vifa MG10SD109-08) placed 1 m above the operant conditioning chamber and calibrated to an output of 70 dB (SPL meter, RION NL 15, RION) at the food hatch. A custom-made operant conditioning chamber controller (ELD, Leiden University) was connected to the fluorescent tube; loudspeaker; pecking keys and food hatch to control the Go/No-Go procedure and register the birds' key pecking behaviour during this procedure.

Food intake was monitored daily and before and after the experiment the birds were weighted to monitor their condition.

Song stimuli construction

We constructed songs consisting of natural zebra finch song elements arranged in particular orders. These elements were selected from our own zebra finch song database, consisting of undirected song. See Holveck *et al.* (2008) for recording specifications.

Six element types occurring in natural zebra finch song were selected to construct the song stimuli ('flat', 'trill', 'downslide', 'high', 'curve' and 'noisy') based on optimal discriminability between the elements (see fig. 4.1).

See van Heijningen *et al*. (2009) for a description of these elements (except for the 'curve' type of element). All elements were ramped (3 ms) and rms (0.1) equalized in PRAAT (version 5.1.15, freeware available at www.praat.org).

Song stimuli were constructed with 40 ms silence in between consecutive elements to form song structures like ABA and BAB. Furthermore, we added 50 ms silence at the start and end of each song to avoid acoustic distortions during playback from the loudspeaker.

To avoid pseudo-replication and order effects, each bird was exposed to an individual combination of element types. So for instance, the song structure ABA was for one bird consisting of 'flat-downslide-flat' while for another bird this was 'high-trill-high' and so on (see fig. 4.1 for examples).

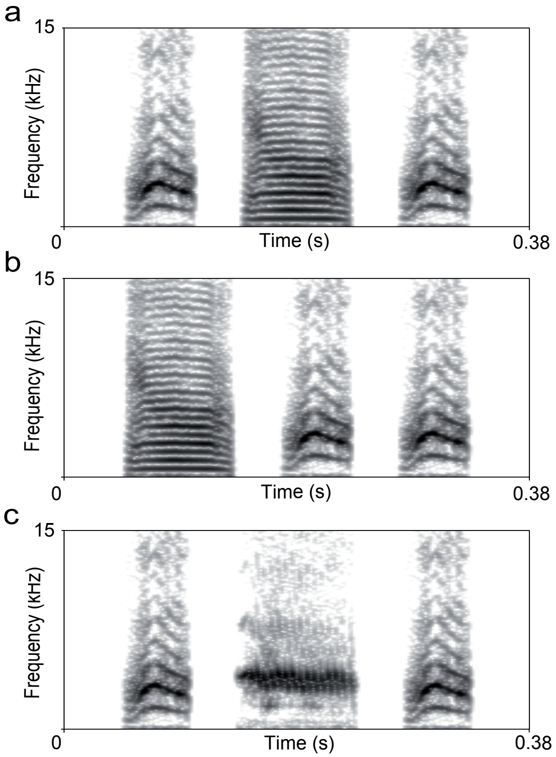

Fig. 4.1. Spectrogram examples of constructed song stimuli. **a**) Curves and stacks in ABA order. **b**) Stacks and curves in BAA order. **c**) Curves and trills in ACA order.

Go/No-Go procedure

To test zebra finches whether they are able to discriminate between acoustic stimuli with an XYX structure against stimuli with an XXY and XYY structure, they were trained in a Go/No-Go procedure with food as a reward. In order to learn the Go/No-Go procedure, they were pre-trained with a natural song from the same database and a 2 kHz tone (equalized on rms 0.1 and ramped with 3 ms) of equal length (0.58 s) constructed in PRAAT.

A peck on the left sensor when its LED is on resulted in a sound stimulus. In 50% of all cases this sound was a ‘Go’ stimulus (S^+, the natural zebra finch song motif) after which the bird had to peck the then LED illuminated right sensor within 6 s. Subsequently, the food hatch opened for 10 s and the bird was able to eat as a reward. In the other 50% of the cases (in randomized order) that the bird pecked the left sensor, a ‘No-Go’ sound (S^-, the tone) was played. If the bird subsequently pecked the right sensor within 6 s, the light was switched off for 15 s. For three birds, this period was prolonged with a maximum of 15 s to promote discrimination learning. For a more detailed description of the Go/No-

Go procedure, see van Heijningen *et al*. (2009).
After reaching criterion performance (>75% response after hearing S^+ and < 25% response after hearing S^-, on at least two consecutive days) the bird entered the next stage in the experiment (transfer on the third day). A DR (discrimination ratio, calculated as $S^+/(S^+ + S^-)$) larger than 0.75 was taken as an alternative learning criterion if a positive or negative response bias prevented some birds from reaching the separate criteria for S^+ or S^-.

General outline of the experiments

After pre-training the birds with the song and the tone, the first experiment examined whether zebra finches are able to discriminate three-element stimuli in which the first element is the same as the last element (ABA and BAB - the positive stimuli) from stimuli in which they were different (BAA, AAB, BAA and ABB - negative stimuli). For this experiment, the pre-training stimuli were abruptly replaced by the experimental stimuli. The overall ratio of positive and negative sounds played was 50/50. At the end of this experiment (when the criterion for reaching discrimination had been reached again), probe sounds were used to assess which of several potential criteria the birds might have used to discriminate the training sets. Part of the birds were next subjected to a second experiment, consisting of a training phase in which the stimulus set used in experiment 1 was extended with additional stimuli (see below for details), again being followed by a test phase using probe sounds.

Experiment 1: Can zebra finches learn to discriminate ABA and BAB from AAB, BBA, BAA and ABB?

Eight out of ten birds learned to distinguish ABA and BAB from ABB, BAA, BBA and AAB (average 24975 trials, ± 5185 SEM/range: 6956-47513, n=8). It is already known (Verzijden *et al*. 2007; van Heijningen *et al*. 2009) that zebra finches are sensitive to element sequence in acoustic stimuli. The current results confirm that conclusion and demonstrate that a songbird species can learn to distinguish this type of three-element stimulus sets.

Experiment 1 revisited: How are the training sets distinguished?

The crucial question about the outcome of the training is *how* the birds solved the task and, more in particular, whether this is by applying some abstract rule. Note that the composition of the stimulus sets does preclude some rules or some simple cues from being used. For instance, using the presence or absence of an AB- (or BA)-bigram does not allow discrimination of the two sets, nor does learning about the absolute position an A or B-element, attending to the first or last two elements of the sequences only, or the number of A's and B's present in a stimulus.

Nevertheless, there are still multiple ways of discriminating between the sets. One rule would be: ‘If the first and last element of a sequence are the same (independent of sequence length and types of element used): Go, and if they are different: No-Go’. A variant might be to learn the structure rule either for the Go or for the No-Go stimuli and to treat every other stimulus as belonging to the other category. Other rules might be: ‘If the stimulus contains no repeated element: Go, and if it does, treat it as a No-Go’, or: ‘If the stimulus contains two transitions between different element types (i.e. both AB and BA): Go; if it has one transition between types: No-Go’ (again, here also variants can be to learn a structure rule for one set of stimuli and ignore the structure of the others). Apart from using a rule, however, the discrimination can also be done by memorizing the individual sequences, either all six, or alternatively just the ones connected to the S^+ or those connected to the S^- without recognizing the structural similarities among S^+ or S^- stimuli.

Methods

In order to find out how the birds discriminated between the stimuli, we presented them with ‘probe’ songs. These probe songs were not reinforced to avoid any additional learning and intermixed with the already familiar training stimuli in 20% of stimuli presentations and presented at least 40 times each in shuffled order (random without replacement). Also, ‘control’ probes identical to the training stimuli were added to control for the effect of non-reinforcement.

We did two consecutive probe tests. The first comprised of the probe stimuli ACA, CAC, ABBA and BAAB (C being a novel element type), together with probe (non-reinforced) versions of the positive training stimuli. If the birds learned the ‘first and last identical’ rule, a response to ACA and CAC indicates that the rule was generalized to new element types, as ‘C’ is yet unknown to them. A more restricted version of the rule would be ‘first and last identical’ but only for stimuli consisting of A’s and B’s. Applying this rule would result in treating ACA as familiar and CAC as a novel, and initially neutral stimulus. Probing with ABBA and BAAB would in this case be predicted to give the same response as to ABA and BAB (as the length of the sequence is irrelevant). Alternatively, if the birds were responding to the presence of repetitions, ABBA and BAAB should be treated as S^- stimuli.

The second probe test contained ABAB, BABA, AABB and BBAA, together with (control) probe versions of the negative training stimuli. In case the birds have learned ‘first and last different’ for the S^-, they should not respond to these probes. Sensitivity to transitions between subsequent elements (is the same as ‘absence of repeats’) or to presence of repeats should make them treat the

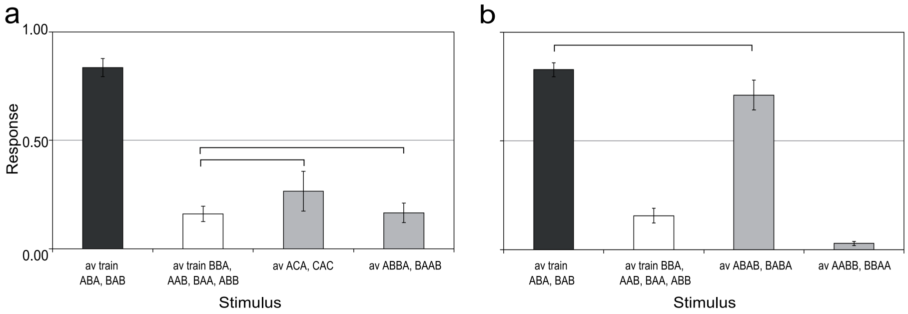

Fig. 4.2. Averaged response patterns (n=8) during the first (a) and second (a) probe test (± SEM) of experiment 1. Black bars indicate positive training stimuli; white bars negative training stimuli and grey bars probe stimuli. Lines indicate homogenous response following G-tests of independence between training stimuli and between training and probe stimuli (Sokal and Rohlf 1995) with X^2 critical value of 9.488 ($p<0.05$).

ABAB and BABA stimuli as positive ones, and AABB and BBAA as negative ones.

Results

The response pattern for the two probe tests are given in fig. 4.2. We applied Simultaneous Testing Procedures (STP) based on G-tests of independence to assess which stimuli were responded to similarly ($p< 0.05$). A Williams correction was calculated when numbers were lower than 5 but this never changed the outcome (Sokal and Rohlf 1995) based on the averages of training- and test stimuli sets with a similar structure. After reaching learning criterion, the responses to ABA and BAB were not homogeneous with the responses to ABB, BAA, BBA and AAB during both probe tests (see fig. 4.2). Responses to the probe version of ABA and BAB during the days of the first probe test and to the probe versions of BBA, ABB, BAA and AAB during the second probe test were homogeneous compared to the training (reinforced) version of these structures, except for ABA of bird 822 (fig. 4.2 and 4.3a). We therefore compared all 'new' probes with the responses to the training sounds.

The responses to the ACA and CAC probes did, at group level, not differ from those to the S^- stimuli, showing that the birds were not generalizing from their training to new element types arranged in a similar structure as the positive stimuli. At the individual level however, one bird (822, see fig. 4.3a) responded to both ACA and CAC in the same way as to ABA and BAB. Three birds treated ACA and CAC as neutral, as their response was intermediate compared to both sets.

At group level, the response to the ABBA and BAAB probes was not similar to ABA and BAB. Instead, they were similar to the S^- training set. Five individual birds followed this pattern, while three birds differed from both S^- and S^+.

In the second probe test ABAB and BABA were treated similarly to the positive training set ABA and BAB on group level (see fig. 4.2b). On individual level however, these probes were treated as neutral by four birds, as the response was in between the positive and the negative training stimuli.

At group level, AABB and BBAA differed from both positive and negative training sets. However, this was not due to an intermediate response towards these probes, but to an even lower response to the S^- stimuli. This pattern was present in five individuals, of which three never responded to these probes.

A special case is bird 822. As mentioned above, in contrast to the other birds, he responded positively to the ACA and CAC songs (fig. 4.3a) and could therefore have learned a rule not linked to the identity of A and B. We therefore designed a third probe test to explore which rule this bird might be using. This set contained stimuli involving a novel element type (D), as well as probes with multiple transitions. It can be observed, see fig. 4.3b, that 822 responded to the new probes CADC and ACDC similarly compared to the positive training set; intermediate to ADA, ADD and ACBCA and more similar to the negative training set to ABBBA. These results suggest that 822 responded positively to stimuli with new element types, in particular when these had transitions between element types. ABBBA seemed to be perceived as a compound negative stimulus (ABB and BBA). There was no clear pattern however corresponding to the use of a particular rule. (As no clear pattern could be discerned in the responses in the third probe test, no G-tests were performed.)

Discussion

Together the above results allow exclusion of some potential rules. First, if the birds learned a rule, it was linked to the presence of A and B elements and not transferred to novel elements (with the possible exception of bird 822). Secondly, the positive response to ABAB and BABA and the negative response to ABBA, BAAB, AABB and BBAA show that the birds do not use the ‘first and last identical’ or ‘first and last different’ rule. Most birds did not treat the probes as neutral, but differentiated among them, classifying them with, respectively, S^+ or S^- stimuli.

One explanation might be that the birds (excluding 822) score the probes according to whether these contain the exact three element sequences from the training and not by an abstract rule (S^+ stimuli present in: ABAB, BABA; S^- present in: BAAB, ABBA, AABB, BBAA). An alternative hypothesis is that they were sensitive to the presence/absence of repeats (bound to A’s and B’s). This fits with the observation that AABB and BBAA, each containing two repeated elements, are treated as ‘super negative’ stimulus.

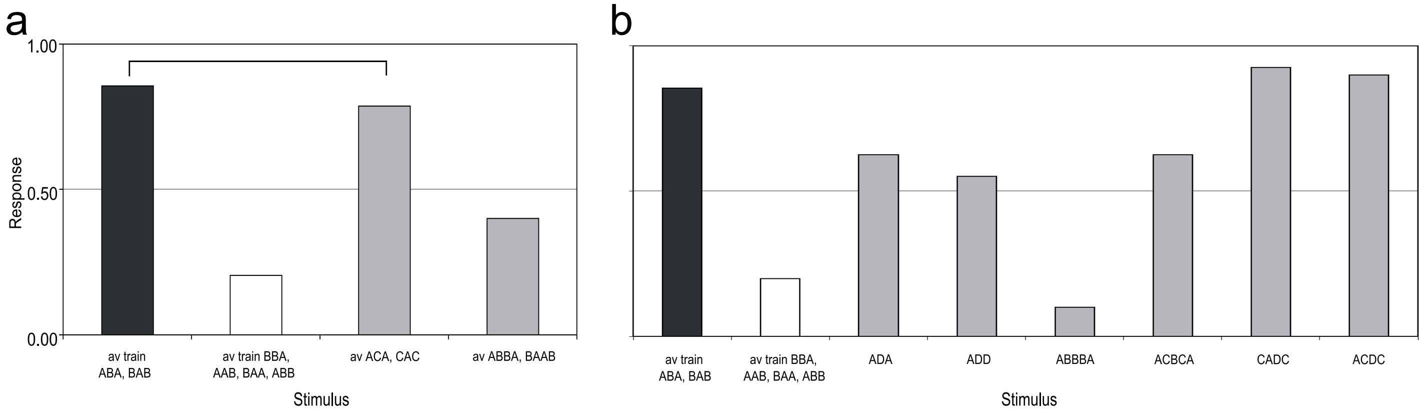

Fig. 4.3. Response patterns to the first (a) and third (b) probe test of bird 822 of experiment 1. Black bars indicate positive training stimuli; white bars negative training stimuli and grey bars probe stimuli. Lines indicate homogenous response following G-tests of independence between training stimuli and between training and probe stimuli (X^2 critical value of 9.488, $p<0.05$). **a**) Only the responses of 822 to ACA and CAC were homogeneous to ABA and BAB. **b**) As no clear pattern could be discerned in the responses in the third probe test, no G-tests were performed.

Experiment 2: How do zebra finches cope with an extension of the training set?

To further explore the rules that the birds may have used to distinguish the training sets and also to see whether these rules can be altered if additional training sounds cannot be classified correctly with these rules, we extended the training.

Methods

In this experiment we added ABAB, BABA, AABB and BBAA as S^- stimuli, and ABBA and BAAB as S^+ stimuli. If the birds used a rule as 'presence/absence of repeats', they can no longer use it to distinguish between the sets. Also, counting the number of transitions between different elements will not result in properly classifying the new ones. However, all S^+ stimuli still have a 'first and last identical' structure and all negative S^- a 'first and last different' structure.

Results

All birds were subjected to new training sets. We noted that in the first days after switching to this set the birds seemed to change their responses to the original training sounds (ABA, BAB, ABB, BBA, AAB, and BAA) even though these remained to be reinforced in the same way as before. Table 1 shows the responses to the original training stimuli just before and over the first nine days after introduction of the additional training sets in more detail. On day one after transfer, the averaged response to the familiar negative stimuli, consisting of three

Table 4.1. Reponses and Discrimination Ratios (DR) to the original training stimuli before and after transfer to experiment 2.

Days after transfer	-2*			1			4			9		
Bird ID	**S^+**	**S^-**	**DR**	**S^+**	**S^-**	**DR**	**S^+**	**S^-**	**DR**	**S^+**	**S^-**	**DR**
778	0.75	0.10	0.88	0.57	0.03	0.95	0.68	0.16	0.81	0.71	0.19	0.79
779	0.89	0.11	0.89	0.88	0.19	0.83	0.87	0.50	0.63	0.93	0.51	0.65
796	0.65	0.12	0.84	0.73	0.10	0.88	0.85	0.51	0.62	0.95	0.79	0.55
813	0.90	0.16	0.85	0.93	0.54	0.63	0.97	0.86	0.53	0.98	0.74	0.57
825	0.76	0.02	0.98	0.50	0.04	0.93	0.70	0.31	0.70	0.60	0.23	0.72
820	0.82	0.14	0.85	0.80	0.18	0.81	0.72	0.35	0.67	0.84	0.59	0.59
822	0.90	0.17	0.84	0.81	0.14	0.85	0.95	0.42	0.69	0.89	0.64	0.58
844	0.78	0.09	0.90	0.95	0.56	0.63	0.85	0.63	0.57	0.86	0.72	0.54
average	0.81	0.12	0.88	0.77	0.22	0.81	0.82	0.47	0.65	0.84	0.55	0.62

* data of day -2 contains the averaged data over the last 2 days before transfer

elements: ABB, ABB, BAA and BBA, was under 0.25. Over time however, this value increased to on average 0.55 on day nine. The average of the positive three element stimuli, ABA and BAB remained above 0.75. This might indicate that the birds did not base their discrimination on the individual stimuli separately, but may have used some more general 'rule', such as 'presence (or absence) of repeats', which they stopped using after transfer to learn a new way to discriminate between these larger sets.

For logistic reasons, we could continue this training for only five birds. Over time, two of these five birds that we trained with this larger set reached the overall criterion performance after 36112 and 52474 trials respectively (in 151 and 107 days respectively.). Two other birds did not improve their level of discrimination and we stopped training them. One bird was taken out for health reasons.

Interestingly, the two birds that succeeded in the second discrimination training were relatively quick in improving their DR for the three-element stimuli immediately after the start of the new training phase (0.65 and 0.72 on day 9), compared to the other birds (0.55, 0.57 and 0.59) suggesting that the speed of learning indicates later success.

As there are still several strategies the birds could have used to discriminate between the sets we again performed probe tests. Again, the crucial question after this new training is *how* the birds solved this task.

Experiment 2 revisited: How are the extended training sets distinguished?

Compared to the previous probe tests, the current task can not be resolved by attending to repeated elements, as both the training sets contained repeats and hence these are not indicative anymore. The same holds for attending to transitions. As ABAB and BABA in the negative set also consist of a series of transitions, this cannot be used anymore as an indicator of a positive stimulus. However, there are still a number of ways to discriminate between the sets. One of them still is to compare the first and last element of the sequences (first and last identical). Alternatively, they can discriminate the stimulus sets by rote memorization of the individual stimuli.

Methods

To uncover how the birds discriminated between the extended stimulus sets, we again gave them two consecutive probe tests. The first test included ACA, CAC, ABAA, AABA, AABBA, ABBAB and control probes ABA, BAAB, AAB and ABAB. ACA and CAC were tested (again) as a control for whether they would now generalize to new element types. ABAA and AABA both contain one positive (ABA) and one negative sequence (BAA and AAB respectively). A positive response could indicate the use of the 'first and last identical' rule that they could now also apply to four element songs. But it could also indicate that the bird was paying attention to presence of ABA in the total sequence and discarding that it also contained a negative one (BAA or AAB). A negative response might be due to focus on presence of an S^- stimulus in the novel stimulus. AABBA holds more negative than positive stimuli (positive: ABBA, negative: AABB, AAB, ABB and BBA) but has an overall first and last identical structure. In case the birds learned the 'first and last identical' rule they should respond positively to AABBA. Subsequently, they should respond negatively to ABBAB, but if the birds paid attention to the presence of ABBA and/or BAB instead of a more general rule, they were expected to respond positively.

In the second probe test, we tested the response to AAA, ACCA, CAAC, ACAC, CACA, DCD, DDC and CDD (ABA and AAB were added as control probe sequences). AAA is an example of a non-transition song, but the first is the same as the last element. The responses to the other probe songs indicate whether the birds can generalize what they learned to new element types, as the birds have not been trained before with C's and D's.

Results

See fig. 4.4 for the individual response patterns during the probe tests for birds 825 and 779. G-tests confirmed that each control probe was homogeneous with its reinforced version. Analogous to the analysis in experiment 1, we compared the new probes to the reinforced training stimuli.

Further G-tests showed that the S^+ and S^- set were not homogeneous, as expected from the fact that the birds reached the overall learning criterion. Next, we tested whether the responses to the subsets (ABA and BAB compared to ABBA and BAAB etcetera) within the training sets were homogenous for each bird. This was the case for one bird only (825, see fig. 4.4b and d). For this bird all new probes were homogenous with the negative training set average (BBA, ABB, AAB, BAA, ABAB, BABA, AABB and BBAA) for both probe tests. For the other bird (779), the within-training sets comparisons were not homogeneous and could therefore not be averaged (see fig. 4.4a and c). The score for ABBA and BAAB was high, but not as high as for ABA and BAB (0.74 compared to 0.93 respectively). Of the negative training stimuli, only AABB and BBAA were homogeneous with the negative three element training stimuli (but only during the second probe test), ABAB and BABA were not. This indicates that the bird did not acquire a general underlying criterion based on the structure of the songs to distinguish the S^+ and S^- sets. It is, however, discernable that this bird responded very little to all probes including novel element types (C or D) and to the AAA probe, but showed intermediate responses to AABA, AABBA and ABBAB.

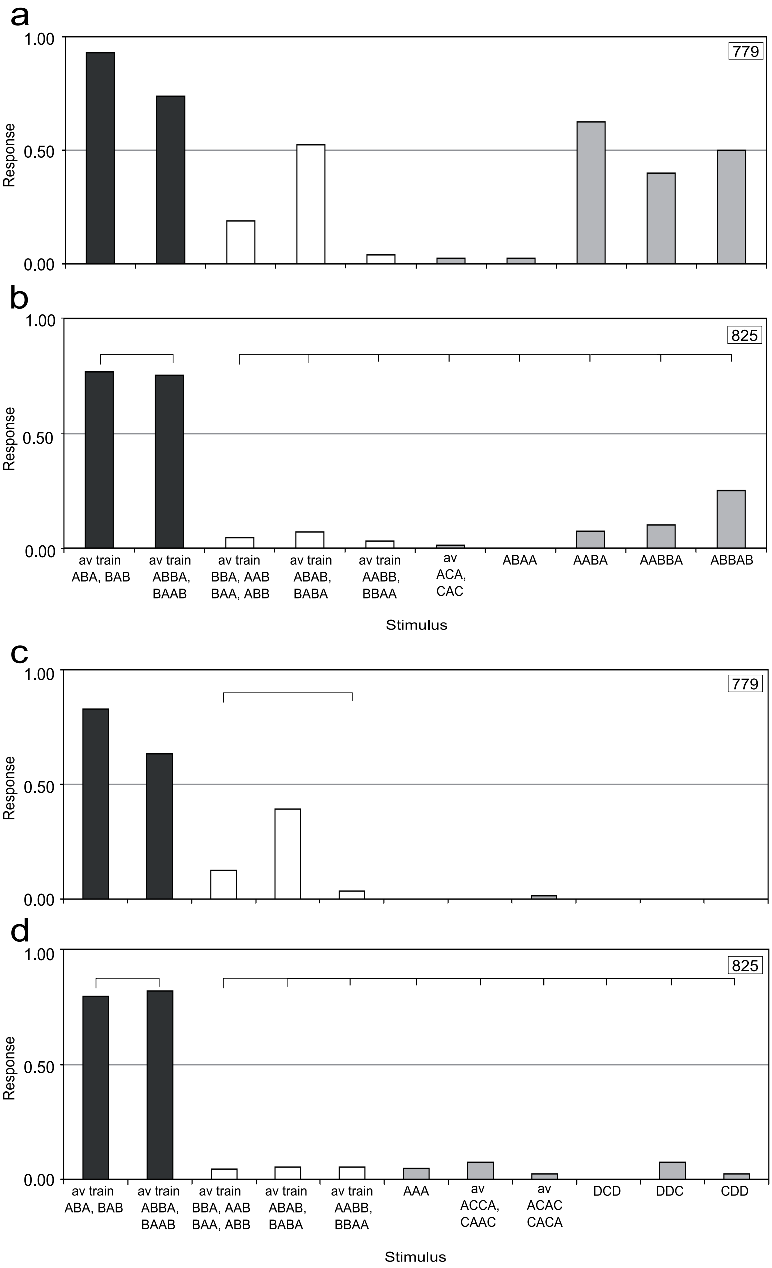

Fig. 4.4. Response patterns to the first (a and c) and second (b and d) probe test of experiment 2 of birds 779 and 825 respectively. Black bars indicate positive training stimuli; white bars negative training stimuli and grey bars probe stimuli. Lines indicate homogenous response following G-tests of independence between training stimuli and between training and probe stimuli (X^2 critical value of 22.362, $p<0.05$).

Discussion

What is clear from the results above is that both birds did not generalize to new element types as for instance the responses to all songs with C's or C's and D's were low, also to those resembling positive songs in overall structure (ACA and ACCA). What also holds for both birds is that they did not use a comparison between the first and last element, as they responded positively or ambiguous to probes that should be classified as negative according to this rule.

The most likely interpretation of the response pattern to the new probes for bird 825 (all negative) is that he learned to respond to the individual positive training sequences and rejected every other sequence, also novel ones.

The interpretation for 779 is slightly different. She clearly discarded probes with novel element types, even those similar in structure to the positive training stimuli. However, she responded more ambiguous to AABA, AABBA and ABBAB, while also having an intermediate response to the training stimuli ABAB, BABA, ABBA and BAAB. This might be explained by the fact that these training stimuli and probes contained three-element sequences that in themselves were reinforced in the opposite direction compared to the full sequence. Taken together, 779 most likely paid attention to both the full sequences as well as to the presence of positive and negative three element training stimuli in the probe sets.

GENERAL DISCUSSION

Our data demonstrate that zebra finches can learn to discriminate ABA, BAB from BBA, BAA, ABB and AAB. At first sight such discrimination may suggest that the birds obtained some sort of insight in the abstract rules differentiating the stimulus sets (i.e. learning of an XYX vs. an XYY or XXY structure, where X and Y can be any element type). Nevertheless, demonstrating such an ability requires two types of tests. The first one, and the one most commonly applied in AGL experiments, is whether training with one set of tokens (in this case A and B-elements) induces generalization to a novel set (C and D-elements), analogous to a 'new vocabulary' in human language (Marcus *et al*. 1999; Marcus 2006; Frank *et al*. 2009). Such a generalization test has been done for instance in tamarins (Endress *et al.* 2009a) but most experiments focused on generalization to new tokens of familiar types (i.e. replacing a specific 'A' element by another 'A') or new combinations of familiar types (i.e. Newport *et al*. 2004; Gentner *et al*. 2006; Herbranson and Shimp 2008; Hauser and Glynn 2009; Abe and Watanabe 2011). Only this study, van Heijningen *et al*. (2009) and Murphy *et al*. (2008) tested generalization with sequences of new element types (although in this last study the novelty of these stimuli was questioned by Corballis, 2009)).

In our experiment only one out of eight birds seemed to generalize to novel elements and treated ACA and CAC similar to ABA and BAB. This result is comparable to that we obtained in our previous study (van Heijningen *et al*. 2009) in which we showed that zebra finches had no difficulty in generalizing to novel exemplars of the same element type, and in which also only one bird transferred a learned discrimination to a novel element type set. It shows that zebra finches have the potential for such a generalization, but don't use this automatically. The current experiment was, however, also not aiming to see whether this ability could be specifically trained.

The second type of test to apply after mastering an XYX vs. XXY or XYY distinction is for how the distinction is made. Probe tests are essential for this, as has also been demonstrated by Gentner *et al*. (2006) and van Heijningen *et al*. (2009). Van Heijningen *et al*. (2009) showed that the birds relied on simple, not rule based, strategies when possible. That study also demonstrated individual variation in strategies, as has also been shown in humans in AGL studies (Zimmerer *et al*. 2011). The current study showed no evidence of learning a rule like 'first and last elements are similar vs. dissimilar'. A possible 'rule' that the birds learned after the first training is to attend to presence or absence of repeats – these were present in all negative stimuli and in the probes treated as such, and absent in all positive training stimuli. Alternatively they might have attended to the presence or absence of the specific training sequences. In this respect it is of interest to compare the responses to the XXYY and XYYX stimuli. Both probes contain two S^- stimuli, but XXYY gets fewer responses than XYYX. As XXYY contains two repeats, one more than the S^- stimuli, the more extreme responses to this structure might indicate a peak shift phenomenon: responding stronger to a novel stimulus that overemphasizes the characteristics used to discriminate the S^+ from the S^- (cf. Verzijden *et al*. 2006). This 'supernormal' response therefore supports the hypothesis that the most likely rule the birds used might be 'presence of repeats' compared to presence of any two training stimuli. No longer being able to rely on presence/absence of repeats may also explain the confusion after switching to Experiment 2.

Also in van Heijningen *et al*. (2009) we found that the simpler rules the zebra finches used included repeats. Interestingly, as already mentioned in the introduction, repeats have previously been suggested to be important POMPs in humans (Endress *et al*. 2009a). These Perceptual Or Memory Primitives, like sensitivity to edges of strings or repeated units may serve an important role in early learning and are suggested to be a basis for the evolution of linguistic learning mechanisms (Endress *et al*. 2009b). Our study suggests that not just humans and chimpanzees, but also other animal species might be sensitive to POMPs.

In Experiment 2 we extended the training set so that the presence or absence of repetitions could no longer be used to discriminate between the sets. The rapid loss of discrimination among the 3-element stimuli and difficulties in mastering the new discrimination demonstrates that the birds abandoned one type of rule ('repeats'), but struggled to replace it by a new one. Neither of the two birds succeeding in the discrimination solved it by attending to the regularity of 'first and last identical vs. different'.

We want to emphasize that experiments like ours can never prove that zebra finches (or any other species) are unable to learn and apply a more abstract rule, and hence leave open the question whether they might be able to do so in a different paradigm or problem. Using a different paradigm, a study of Bengalese finches, a relative of the zebra finch, suggests they may have some more complex rule learning ability (Abe and Watanabe 2011), but see also Yamazaki *et al* (2011) on another recent rule learning study in Bengalese finches with non-acoustic stimuli. Hence it can be that a different training paradigm might also result in abstract rule learning in zebra finches. Also, our results highlight that certain types of regularities are being noticed and used for generalization to novel stimuli. However, given that the zebra finches did readily master the basic training by using simple regularities of the training sets, rather than more abstract rules, our study demonstrates the need for extensive and critical probe testing in AGL experiments to detect what these regularities might be, and also to examine whether these differ among individuals. Finally, we suggest that our results might also indicate that *human* experiments may benefit from a wider use of testing stimuli to analyze the actual rules used by humans to solve AGL tasks.

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ETHICAL STANDARDS

This study was conducted according to the Association for the Study of Animal Behavior guidelines on animal experimentation as well as to the Dutch law on animal experimentation. The Leiden committee for animal experimentation (DEC) approved the experiment under number 09228.

Chapter 5

Thesis overview and synthesis

In this thesis I explored both the natural variation in zebra finch song as well as the perceptual abilities of zebra finches to detect syntactical regularities in artificial acoustic stimuli, specifically addressing the questions raised in the debate on artificial language learning in animals.

Chapter 2 ('Zebra finch phonology and phonological syntax across populations and continents') gives an overview of the natural variation in phonology (element types) and phonological syntax of zebra finches. We collected songs of 13 zebra finch populations worldwide, distributed over three continents (North America, Europe and Australia). By using a recently developed software program (Luscinia) we measured and calculated multiple acoustic characteristics, leading to a clustering solution of 11 element types. All these 11 element types occur in all populations, but the populations (and to a lesser extent also continents) differed significantly in the relative frequencies of these element types. In terms of relative position in the song, we observed that two clusters tend to occur in the end part of the song, species-wide, in many populations and all continents, although Australia in this case shows a slightly different pattern. We also found significant differences in the total number of elements per song between the labs but not between the continents.

We did not find evidence for structural constraints in the order of elements in natural zebra finch song on a species-wide scale, nor on continent level. However, we did find evidence for the presence of structural constraints in 8 out of 13 populations. These results shed light on the origin of variation in song characteristics between populations of this widely used study species for language evolution and vocal learning. Moreover, they are suggestive of cultural differentiation being at least as important as genetic diversification processes in causing large scale changes in song characteristics.

The other two chapters in this thesis are about the perception of syntactic rules by zebra finches.

Chapter 3 ('Simple rules can explain the discrimination of putative recursive syntactic structures by a songbird species') describes the results from a Go/No-Go experiment that was designed to test whether zebra finches have the ability to distinguish an X^nY^n from an $(XY)^n$ structure (where X and Y stand for any type of element). This experiment examined the questions raised in the debate on artificial rule learning in animals concerning both the generalization to new element types and the need for additional tests (probe tests) to examine the actual rules that the birds used to make the distinction. Probe stimuli were stimuli that were not reinforced, so that there was no additional learning due to the exposure to these stimuli. Because they were not reinforced they were presented in only 20% of all stimuli presentations in order to avoid extinction.

The stimuli structures were examples generated by a relatively complex Context Free Grammar (a specific type of Phrase Structure Grammar) and the simpler Finite State Grammar, respectively. Fitch and Hauser (2004) used this contrast between these grammars to find out whether animals, in their case cotton-top tamarins, can discriminate between sequences from these two grammars that according to Hauser *et al*. (2002a) might be the characteristic that makes human language unique. Fitch and Hauser (2004) concluded that only the human subjects in the experiment had this ability, not the tamarins, confirming the proposition made by Hauser *et al.* (2002a). Some years later though, this conclusion was challenged by Gentner *et al*. (2006) who claimed that starlings, a songbird species, *could* discriminate between song sequences following the FSG and CFG grammar. Apart from the ongoing debate whether the CFG stimuli used were actually reflecting hierarchical sequences (or 'recursion', as the authors labeled these sequences), these claims were controversial and preliminary, as a generalization test with new element types was still absent and the test results for the use of less complex rules than a hierarchical,'recursive' rule were ambiguous. In our study, using the zebra finches, we did a comparable experiment, but extended it with the further training and testing needed to unambiguously answer the question whether zebra finches can discriminate between the 'recursive' and 'non-recursive' song structures as used by Fitch and Hauser (2004) and Gentner *et al*. (2006). We subsequently showed that zebra finches, when trained and tested in a Go/No-Go experiment similar to starlings, were able to generalize the distinction between XYXY and XXYY to stimuli constructed using novel elements belonging to the same categories (for instance, element type A contains exemplars a_1, a_2 etc.) . However, 7 out of 8 birds failed to generalize to unfamiliar element categories, an essential test that was not performed in the starlings (from songs with A's and B's to songs with C's and D's). This suggests that the acquired discrimination in zebra finches was based on phonetic rather than syntactic generalization. The eighth bird, however, must have used more abstract, structural, cues. Nevertheless, further probe testing showed that the results of this bird, as well as those of others, could be explained by more simple rules than 'recursive' ones.

In the final chapter, **Chapter 4, ('Rule learning by zebra finches in an artificial language learning task: which rule?')** we again trained and tested zebra finches in a Go/No-Go experiment. This time we tested their ability to distinguish different three-element sequences based on their abstract arrangement and further examined which rule they used to do this. This XYX versus XXY and YXX (in which X and Y again can be any element type) contrast was tested in infants (Marcus *et al*. 1999) as an example of a more general cognitive rule (the first and last element of a sequence are identical) that infants can use in language learning. Our setup addressed questions raised after the AGL experiments by Marcus *et al*. (1999) and those of Murphy *et al*. (2008) concerning the ability of animals to learn the rule and to generalize it to new element types and if so, by which rules. We found that eight out of ten zebra finches learned to distinguish XYX from XXY and YXX sequences. Tests with unfamiliar probe sounds showed that only one bird generalized the discrimination to a new element type. Other probes suggested that discrimination between the stimulus sets resulted from attending to the presence or absence of repeated elements. We continued the training by adding four-element songs following a 'first and last identical vs. different' rule that could not be solved by attending to repetitions. Only two out of five birds learned the overall discrimination. Testing with novel probes demonstrated that discrimination was not based on using the 'first and last identical' rule, but by attending to the presence or absence of the individual training stimuli. The two birds differed in the strategies used. Our results thus demonstrate only a limited degree of rule learning, and also highlight the need for extensive and critical probe testing to examine the rules that animals (and humans) follow in AGL tasks. They also show that rule learning strategies may differ between individuals.

Synthesis

While we found that natural zebra finch song contains relatively little structural constraints or rules on an inter-individual level, we did find that zebra finches were capable of detecting differences in song element order in (artificial) zebra finch song. Some zebra finches were even able to generalize what they learned to new element types, a characteristic that is one of the hallmarks of human language. At first sight this might seem conflicting, but it might be that these rule learning/ detection mechanisms are more domain-general and not restricted to vocal production and perception. They might also be involved in other behaviours than song perception and production, like for instance courtship display behaviour, number quantification, social relationships, navigation and foraging (Hauser *et al*. 2002a). Also in humans it was found that AGL tasks can be performed with visual and tonal sequences, outside a direct language context (Saffran *et al*. 1999; Saffran *et al*. 2007) suggesting that these mechanisms may also be more domain-general.

Our experiments also highlight the need for probe tests to analyze which of a multitude of possible rules, was the most likely one that was used by each individual. There is a relatively high variability in the rules used by different birds, even though they were trained with stimuli based on identical grammatical structures. It has been underestimated in the past (also in our own experiments) how difficult it is to design experiments that can unambiguously be solved by only one type of rule.

Apart from the variety in rules that apparently can be used to solve tasks that have a similar level of 'abstraction' or complexity, the birds also seemed to rely on less complex rules to solve the discrimination task. It is more likely that from the viewpoint of what one might call '*cognitive efficiency*' a subject (human or animal) will use a rule or regularity that is sufficient to make the distinction (the simplest one), in stead of a more complex or abstract one. This phenomenon has been observed in AGL tasks with humans as well (Gerken 2006; de Vries *et al*. 2008; Hochmann *et al*. 2008; see Lai and Poletiek 2011 for an alternative learning method). Further research should take this into account in the design of experiments and their conclusions: when a subject does not show the most elaborate rule learning it does not imply that it is unable, but that there might have been no need to do so.

One of the explanations for not finding any evidence of more complex rule learning is the choice of method applied. We used the Go/No-Go paradigm, which forces the birds to show whether they can detect differences in the stimuli. Others have used other methods like habituation/dishabituation (hab/dishab), in which dishabituation to a novel stimulus is taken as evidence that the subject notices a difference (Hauser *et al*. 2002b; Abe and Watanabe 2011; Hauser and Glynn 2009). However, when there is no dishabituation it may be that the subject *can* hear a perceptual difference (Braaten *et al*. 2007), but that it is not relevant enough to show other behaviour. Nor can it be excluded that with training and reinforcement such as with Go/NoGo experiments, subjects can learn the discrimination, where they would not with only passive exposure. Nevertheless, the benefit of the hab/dishab method is that it is far less time consuming than training birds in a Go/No-Go setting and therefore considerably more birds can be tested with this alternative method in order to reach higher statistical power. In the past we have tried to develop a protocol for hab/dishab testing in zebra finches, but this was unsuccessful at the time due to a relatively large heterogeneity in dishabituation behaviour between the birds. However, recently Abe and Watanabe (2011) claimed to have successfully used this method with Bengalese finches and it might therefore be interesting to re-explore this method (but see Beckers *et al*. 2012).

Moreover, in future experiments on artificial language learning (in both humans and animals), more attention should be given to the design of the stimuli sequences to ensure that discrimination between these sequences can be based on one particular rule only, and not by simpler ones. To complement this, each experiment should be followed by probe testing to unambiguously confirm the exact rule that was used.

To summarize, natural zebra finch song contains heterogeneity in the relative distribution of element types across 13 populations worldwide, but compared between individuals it contains relatively little structural constraints or rules in the order of the elements. In contrast, from a perception point of view, zebra finches are able to detect differences in song structure and are able to learn rules to generalize these regularities to new element types in some instances, a hallmark of human language. This ability might seem conflicting with the absence of clear structural rules in their own natural songs, but it might be that these rule learning/detection mechanisms operate in a more general cognitive context, which has also been suggested for some mechanisms found in human artificial language learning.

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Nederlandse samenvatting

Deze Nederlandse samenvatting is voor het bredere publiek geschreven. Voor de leesbaarheid zijn daarom de referenties weggelaten. In hoofdstuk 1 van dit proefschrift zijn de referenties wel opgenomen.

Taal bij de mens

Een van de grootste verschillen tussen de mens en andere diersoorten is 'taal'. Taal stelt ons in staat om met ons denkvermogen uitingen met een bepaalde betekenis te produceren en te begrijpen. Bovendien stelt taal ons in staat om (in theorie) een oneindig aantal zinnen te maken uit een gelimiteerd aantal elementen. Het is daarom voor wetenschappers, maar ook voor het brede publiek een fascinerende vraag hoe precies zo'n complex fenomeen geëvolueerd kan zijn. Men heeft lang gedacht dat *alle* eigenschappen die met taal te maken hebben alleen bij mensen voorkomen. Maar sinds een aantal jaren is gebleken dat steeds meer mechanismen die ten grondslag liggen aan taalproductie en taalperceptie ook aanwezig zijn in dieren. Een voorbeeld hiervan is vocaal leren, waarbij geluiden die individuen produceren, aangepast worden door te leren van andere individuen. Vocaal leren komt behalve in mensen ook voor in zangvogels, kolibries, papegaaien, vleermuizen en walvisachtigen, maar niet bij bijvoorbeeld onze naaste verwanten, de mensapen.

De zoektocht naar welk aspect van taal uniek is voor mensen en dus niet bij dieren voorkomt, kreeg een nieuwe impuls nadat een groep wetenschappers een controversieel artikel schreven in 2002. In dit artikel suggereren zij dat een bepaalde, hiërarchische vorm in grammatica genaamd 'recursie' uniek is voor mensen. Recursie komt in veel verschillende vormen voor: visueel bijvoorbeeld, in de vorm van het 'Droste effect'. Op deze alom bekende cacaodoosje staat een dame die een dienblad vasthoudt. Op dit dienblad staat weer een cacaodoosje waarop hetzelfde plaatje met de dame te zien is. Het plaatje komt dus in zichzelf terug. In de context van grammatica gaat het dan over zinsdelen die in elkaar terug komen. Een voorbeeld hiervan is de volgende zin: 'de zebravinken, **die de onderzoeker testte**, speelden vals'. Hier is de dikgedrukte zin ingebed in de andere zin. In theorie is het mogelijk om met dit type hiërarchische grammatica (meerdere lagen/inbeddingen) oneindig lange zinnen te kunnen maken waarmee oneindig veel verschillende boodschappen gemaakt kunnen worden. Vervolgens wilde men weten of deze relatief complexe grammatica's inderdaad alleen door mensen gebruikt worden en dus uniek zijn voor menselijke taal. De mogelijkheid tot het maken van hiërarchische grammaticale constructies zou dan een ontwikkeling in de evolutie van taal kunnen zijn die alleen bij de mens heeft plaatsgevonden.

Nederlandse samenvatting

De vraag hoe taal geëvolueerd is een van de lastigste vragen in de wetenschap: taal fossiliseert niet en daarom kunnen we geen onderzoek doen bij menselijke fossielen naar wat de verschillen zijn tussen menselijke taal en communicatie bij dieren. Dit kan bijvoorbeeld wel in het onderzoek naar het verschil in schedelinhoud tussen de huidige mens en onze vooroudersoorten. Een andere manier om evolutionair onderzoek te doen is door te onderzoeken of verwante soorten de betreffende eigenschappen wel of niet hebben. Met die informatie kan men afleiden of de gemeenschappelijke voorouder deze eigenschap ook had. Daarom is men gaan onderzoeken in hoeverre onze naaste verwanten, andere mensapen, ook taal hebben. Echter, deze hebben voornamelijk non-verbale communicatie die bovendien niet aangeleerd hoeft te worden en lang niet zo complex en gevarieerd is, zoals dat bij mensen wel het geval is. Vervolgens is men gaan kijken naar andere diersoorten die wel complexe, geleerde, vocale communicatie hebben, waaronder zangvogels. Zangvogels zijn relatief ver aan ons verwant (de laatste gemeenschappelijke voorouder is een pre-reptielachtige). Het is daarom waarschijnlijker dat deze communicatiemechanismen die bij de mens tot taal geleid hebben, meerdere keren onafhankelijk van elkaar zijn ontstaan. Maar, ze kunnen ons wel inzicht geven in gemeenschappelijke onderliggende mechanismen van taal en welke precursors uiteindelijk geresulteerd hebben in de complexe cognitieve systemen die betrokken zijn bij taal.

Om onderzoek te doen naar welke eigenschap menselijke taal uniek maakt, hebben wij gekozen voor de zebravink als modeldier, een zangvogel die erg populair is in neuro- en gedragsbiologisch onderzoek en veel overeenkomsten heeft qua zangontwikkeling in vergelijking met de taalontwikkeling bij de mens.

De overeenkomsten tussen zangontwikkeling bij zangvogels en taalontwikkeling bij mensen.

De functie van vogelzang is het verdedigen van het territorium en het aantrekken van partners. In gematigde streken als Noord-Europa zingen meestal alleen de mannetjes, in de tropen zijn er ook soorten waarbij beide geslachten zingen. Over het algemeen betekent een liedje van hoge kwaliteit dat het ook een mannetje betreft van hoge kwaliteit (een liedje dat bijvoorbeeld complexer, sneller, luider of langer is dan dat van andere mannetjes). De kwaliteit van het liedje dat een mannetje zingt is hierdoor een indicator voor de fitness van dat mannetje, dus of hij veel nakomelingen kan produceren. Het liedje wordt op jonge leeftijd geleerd van de vader (of andere mannetjes in de omgeving).

Er zijn relatief veel overeenkomsten tussen de taalontwikkeling bij mensen en de zangontwikkeling bij zangvogels. In beide gevallen is er op jonge leeftijd een gevoelige periode waarin zang of taal geleerd kan worden. Bovendien worden volwassenen geïmiteerd, wordt er gebabbeld om te oefenen, moeten ze zichzelf kunnen horen, hebben ze een voorkeur voor soorteigen geluiden en zijn er speciale, hiërarchische neurale mechanismen voor vocaal leren. Deze overeenkomsten maken dat vogelzang van alle andere vocale communicatie tussen dieren het meest lijkt op menselijke taal, of in ieder geval het systeem wat het beste te onderzoeken is. Dus met deze vergelijkende methode kunnen we onderzoek doen naar de evolutie van taal en taalontwikkeling waar het niet mogelijk is om experimenten te doen met mensen vanuit een ethisch oogpunt. Experimenten met zangvogels kunnen daarom waardevolle inzichten geven in de ontwikkeling en neurale mechanismen die betrokken zijn bij vocaal leren en vocale productie.

Van veel zangvogelsoorten is de zang in te delen in elementen, zo ook bij de zebravink (*Taeniopygia guttata*). De zebravink is een zangvogelsoort die veel gebruikt wordt in neuro- en gedragsbiologisch onderzoek en het modeldier in dit proefschrift (zie fig. 1). Over de hele wereld zijn er universiteiten die onderzoek doen aan zebravinken en zij hebben daardoor vaak eigen zebravinkenkolonies.

Productie van grammaticale regels (syntax).

Grammatica, of syntax, refereert in de context van menselijke taal naar het belang van de volgorde van elementen voor de betekenis van het geheel. 'Man bijt hond' betekent iets anders dan 'Hond bijt man', ook al bestaan de zinnen uit dezelfde woorden. Tot vandaag de dag is er geen sluitend bewijs dat dieren de betekenis van hun vocalisaties kunnen veranderen door de volgorde van de elementen aan te passen, in ieder geval niet op zo'n complexe manier als mensen dat kunnen.

Syntax in de context van vogelzang gaat over de mate van variabiliteit in de volgorde van elementen of van (sub)liedjes, onafhankelijk van de betekenis van die liedjes of elementen waaruit ze bestaan (fonologische syntax). Er zijn

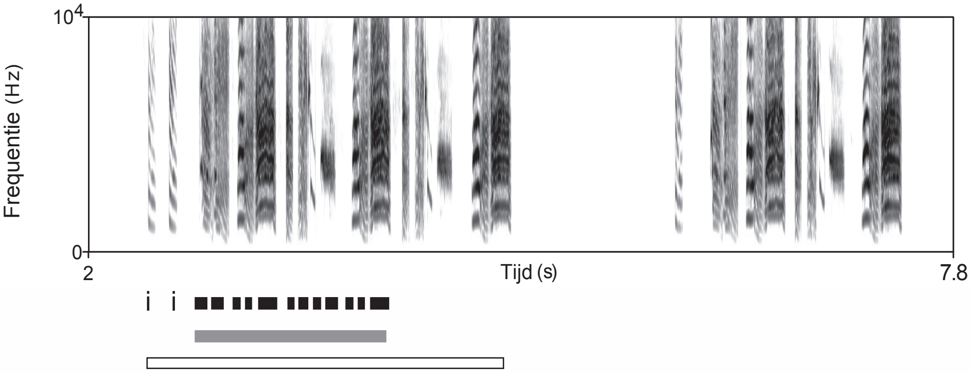

Fig. 1. Voorbeeld van zebravinkenzang. In dit spectrogram (een grafische manier om geluid weer te geven) staat tijd op de x-as en geluidsfrequentie op de y-as. De i's staan voor introductie elementen. De zwarte blokjes staan voor elementen verderop in het lied. De grijze balk geeft een motief aan en de witte balk een motiefreeks. Welke elementtypen voorkomen in het liedje kan tussen zebravinken verschillen.

zangvogelsoorten waarvan een individu maar één element zingt dat herhaald wordt, zoals bij de chipping sparrow, een Noord-Amerikaanse mussensoort (*Spizella passerina*), tot soorten als de nachtegaal (*Luscinia megarhynchos*) die veel variatie laat zien in de volgorde van verschillende (sub)liedjes.

(Natuurlijke) syntax in zebravinkenzang

Er kan op verschillende niveaus gekeken worden naar de mate van variabiliteit in element volgorde (syntax) in zebravinkenzang. Op het individuele niveau is de volgorde van de elementen nagenoeg onveranderlijk. Soms wordt een element weggelaten, herhaald of anders gezongen.

Op het populatieniveau, dus binnen (laboratorium) groepen van zebravinken, is er meer variatie. Ieder dier heeft een subset van alle elementen die voorkomen in die populatie. Ook komen niet alle mogelijke opeenvolgende combinaties van elementen (transities) even vaak voor.

Als men *tussen* populaties van zebravinken gaat vergelijken, blijkt dat er sprake kan zijn van dialecten, al is dat tot nu toe voor een beperkt aantal populaties onderzocht. Omdat zebravinken hun lied leren van andere mannetjes in de groep (en dus niet van mannetjes in andere groepen), kunnen de liedjes tussen populaties steeds meer van elkaar gaan verschillen wat betreft de voorkomende elementtypen en syntax. Dit kan gezien worden als een vorm van culturele diversificatie.

Een van de doelen van dit promotieonderzoek was het doen van een grootschalige, gecomputeriseerde studie naar de natuurlijke variatie in elementen en syntax in zebravinkenzang. Zo'n studie is waardevol voor het (neuro-)biologisch onderzoek naar zang (ontwikkeling) en kan inzicht geven in hoeverre de zang van lab populaties (nog) vergelijkbaar is met elkaar en met die van wilde zebravinken. Bovendien kan het inzicht verschaffen in processen als culturele diversificatie en kan daarom ook interessant zijn voor het onderzoek naar (menselijke) culturele evolutie en het ontstaan van dialecten.

Perceptie en het leren van (syntactische) regels door zebravinken

In een aantal diersoorten (chimpansees, resusapen, penseelaapjes, maar ook bijvoorbeeld ratten en spreeuwen) is gevonden dat ze bepaalde grammaticale patronen of regels die in geluiden kunnen waarnemen, ook al produceren ze dergelijke regels niet in hun natuurlijke vocalisaties. Daarnaast is gebleken dat baby's in ieder geval een aantal van dit soort regels ook al kunnen onderscheiden in spraak, al voordat ze deze regels zelf gaan gebruiken in hun eigen vocalisaties. Er zijn daarom onderzoekers die vermoeden dat dit meer algemene cognitieve mechanismen zijn dan specifieke mechanismen die alleen bij taal betrokken zijn.

Een deel van het dierenonderzoek is afgeleid uit onderzoek naar hoe kinderen regels en patronen in spraak leren herkennen. Dit wordt onderzocht met bijvoorbeeld het blootstellen van baby's aan geluiden waarvan de volgorde is opgebouwd aan de hand van relatief simpele, artificiële grammatica's. Vervolgens worden andere geluiden aangeboden, die volgens een andere syntactische (grammaticale) regel opgebouwd zijn. Als de baby's dan anders reageren, is daaruit af te leiden dat ze het verschil tussen de syntactische regels gehoord hebben. Om te weten te komen of dieren ook dit soort regels kunnen waarnemen zijn zulke experimenten met artificiële grammatica's ook bij dieren gedaan.

Echter, er is vanuit meerdere kanten kritiek gekomen op deze experimenten (bij mens en dier) en de resultaten die eruit voortkwamen. Een aantal hiervan hadden te maken met of het gebruikte geluid soorteigen was of niet; of de geluiden wel echt opgebouwd waren volgens die grammaticale regels; of er ook generalisatie naar nieuwe, onbekende stimuli had plaatsgevonden en ten slotte of er voldoende verificatie had plaatsgevonden van welke regels daadwerkelijk gebruikt waren door de dieren ('probe-tests', zie onder). Door deze punten is het nog steeds de vraag of en tot op welke hoogte, dieren in staat zijn om syntactische regels te onderscheiden.

Mijn proefschrift is daarom ingedeeld in twee delen. Het eerste deel (**Hoofdstuk 2**) gaat over de natuurlijke variatie in elementtypen en syntax in zebravinkenzang. Het tweede deel (**Hoofdstuk 3 en 4**) gaat het over in hoeverre zebravinken in staat zijn syntactische regels te onderscheiden, daarbij rekening houdend met de discussiepunten aan de hand van eerder onderzoek naar perceptie van artificiële talen.

Elementtypen en syntax in zebravinkenzang vergeleken tussen verschillende populaties en verschillende continenten (Hoofdstuk 2)

Dit hoofdstuk geeft een overzicht van de natuurlijke variatie in zebravinkenzang wat betreft elementtypen en syntax. We hebben zang verzameld van 13 zebravinkenpopulaties (waarvan de meeste onderzoekslaboratoria zijn), verspreid over drie continenten (Europa, Noord-Amerika en Australië). Met een recentelijk geschreven softwareprogramma (Luscinia) hebben we een aantal bio-acoustische kenmerken gemeten en berekend, wat resulteerde in een clustering van elementen in 11 typen. Deze 11 elementtypen komen in alle populaties voor, maar er was wel een significant verschil in de relatieve frequenties van deze typen tussen de populaties (en in mindere mate ook tussen de continenten). Wat betreft de relatieve positie van elementtypen in de liedjes (dus of een bepaald elementtype vooral in het begin, midden of het eind voorkomt), vonden we dat er twee typen vaker aan het eind van de liedjes voorkwamen. Dit observeerden we op populatieniveau, en in Noord-Amerika en Europa. Australische liedjes bleken een iets ander patroon te laten zien.

Ook vonden we significante verschillen in het totaal aantal elementen per liedje tussen de populaties, maar niet op continentniveau.

Wanneer we alle liedjes in een keer analyseerden (soortsniveau) vonden geen specifieke syntactische patronen in de zebravinkenzang, noch op continentniveau. Dit houdt in dat alle elementen in min of meer random volgorde voorkwamen, uitgedrukt in een getal waarbij '1' betekent dat er een vaste volgorde is en 0 dat de volgorde 'random' is. Echter, bij analyses op populatieniveau vonden we dat er in 8 van de 13 populaties sprake was van significante syntactische patronen en dat de elementen in deze liedjes dus niet in een random volgorde staan. Deze resultaten laten zien dat er variatie is in zangeigenschappen tussen populaties van deze modelsoort. Bovendien suggereren deze verschillen dat er culturele diversificatie heeft plaatsgevonden in de liedjes van de diverse zebravinkenpopulaties.

Zebravinken gebruikten een ezelsbruggetje om 'recursieve' structuren te kunnen onderscheiden (Hoofdstuk 3)

In dit hoofdstuk hebben we het perceptievermogen van zebravinken getest op het vermogen om een set van meerdere hiërarchische, recursieve AABB liedjes te onderscheiden van een set van niet-hiërarchische ABAB liedjes. Een AABB liedje (of motief) is recursief omdat een AB *in* een AB geplaatst. In de versie zonder recursie is de tweede AB er achter gezet, resulterend in ABAB. Overigens, binnen de (computationele) taalkunde is er discussie of het wel of niet 'hebben' van recursie te testen is met deze AABB- en ABAB-achtige structuren. Hier ga ik echter in deze samenvatting niet dieper op in.

Met een zogenaamd Go/No-Go experiment hebben we geprobeerd om de open vragen in het debat rond het leren van artificiële grammatica's door dieren te beantwoorden. In dit experiment leerde iedere zebravink apart in een zogeheten Skinnerbox te drukken op sensoren (zie fig. 2). Speciaal hiervoor ontworpen apparatuur stuurt het experiment en registreert de reacties van de zebravink. Het leren van de procedure gebeurt met het in kleine stappen handmatig 'shapen' van de vinken, waarbij bijvoorbeeld het dichtbij sensor 1 gaan zitten van de zebravink beloond wordt met voer, daarna alleen nog het echt drukken op de sensor, etc.

Het echte experiment start nadat ze geleerd hebben hoe de Go/No-Go procedure werkt door het leren discrimineren tussen een natuurlijk zebravinkenliedje en een toon. In dit hoofdstuk hebben we onderzocht of ze liedjes met recursie en liedjes zonder recursie konden onderscheiden van elkaar. Er waren eerder een aantal dierenstudies gedaan met penseelaapjes en spreeuwen, maar die lieten zoals hierboven al genoemd, een aantal vragen open. Een van de vragen was of de spreeuwen het ook zouden kunnen met nieuwe elementtypen, aangezien een vereiste van een syntactische regel is dat die onafhankelijk van

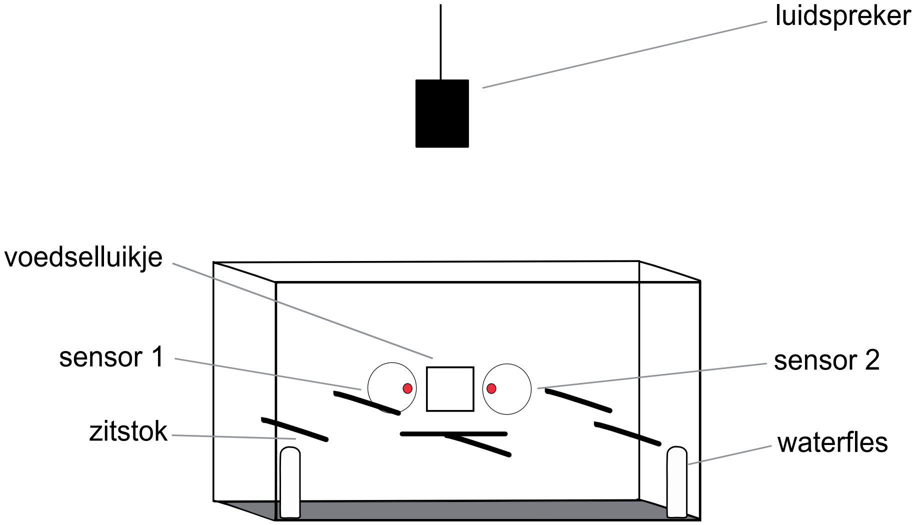

Fig. 2. De Skinnerbox. De Go/No-Go procedure begint met het pikken op de 'jukebox' sensor (sensor 1), waarna er met random kans een of AABB of ABAB te horen is. Afhankelijk daarvan, moet de zebravink dan wel of juist niet op de andere sensor drukken. Als hij er op drukt na het horen van bijvoorbeeld een AABB dan gaat het voerluikje even open en kan hij eten, maar als hij dat ook doet na ABAB, dan gaat het licht even uit (en dat is een lichte vorm van stress). Als hij het verschil tussen deze twee kan horen, zal hij na een aantal keren leren om alleen op sensor 2 te pikken na het horen van AABB en niet na ABAB (om voedsel te krijgen en te vermijden dat het licht uit gaat).

elementtype moet kunnen worden toegepast (dus ook op C's, D's etc). Wat wij vonden is dat ook de zebravinken in staat waren het onderscheid te horen tussen de AABB en ABAB liedjes en dat ze dat ook konden met nieuwe liedjes opgebouwd uit andere A's en B's, net als de spreeuwen. Alleen bleek dat zeven van de acht vogels dat niet meer konden met liedjes die bestonden uit onbekende elementen (C's en D's), de eigenschap die niet getest was in de spreeuwen. Er was echter één vogel (die wij later Zebsky noemden, naar aanleiding van Chomsky, de grote taalkundige), die het onderscheid wel onafhankelijk van elementtype kon maken, dus ook als de liedjes uit C's en D's bestonden.

Een ander nog te beantwoorden punt was of er voldoende verificatie had plaatsgevonden van welke regel het meest waarschijnlijk toegepast was om het onderscheid te kunnen maken. Dit soort taken zijn meestal met meer regels 'op te lossen', dan alleen de onderzochte regel. Het doen van zogenaamde 'probe-tests' kan inzicht geven in welke regels de vogels precies gebruikt hebben om dit onderscheid te maken. In deze probe tests worden liedjes met een bepaalde elementvolgorde aangeboden die niet beloond of 'bestraft' worden en waar de vogels dus niet van kunnen leren hoe ze daar op moeten reageren (bijv. ABBA).

Doordat 'Zebsky' bijvoorbeeld ook op probe-liedje BBBB bleek te reageren, was af te leiden dat hij op de aanwezigheid van een aantal B's aan het eind van het liedje lette, om het onderscheid tussen ABAB en AABB te maken. Bij die probe-tests bleek dus dat ook hij, net als de andere zebravinken, een relatief simpel regeltje (ezelsbruggetje) gebruikte om het onderscheid te maken en niet de relatief complexe recursie-regel, maar die regel wel op een relatief abstracte manier kon toepassen.

Hoe onderscheiden zebravinken liedjes in artificiële taal leerexperimenten? (Hoofdstuk 4)

In het volgende en laatste experiment hebben we ook zebravinken getraind en getest in een Go/No-Go experiment, maar dan om te weten te komen of ze ABA liedjes konden onderscheiden van AAB en BAA (en een uitbreiding van deze set, zie onder). Ook bij baby's en ratten is dit vermogen al eens onderzocht. Bij ratten is dit gedaan zonder ook te testen of ze dat onafhankelijk van elementtype konden en zonder uitgebreide probe-tests voor verificatie van de meest waarschijnlijk geleerde regel om het onderscheid te kunnen maken. Een van de mogelijke regels om dit te doen heet in het Engels de 'non-adjacent dependency rule'. Hierbij is er een verband tussen elementen die *niet* naast elkaar staan. In menselijke taalontwikkeling is deze belangrijk om bijvoorbeeld te leren dat er een verband is tussen de dikgedrukte delen in 'de **zebravink** luister**t** naar een liedje'.

We hebben gevonden dat acht van de tien zebravinken dit onderscheid konden maken, maar na het doen van probe-tests bleek dat maar een van deze vogels dit ook met nieuwe elementtypen kon. Bovendien bleek uit de reacties op andere probes dat de zebravinken het aan- of afwezig zijn van herhaalde elementen gebruikten om het onderscheid te maken. Voor de zebravink die op ACA reageerde bleek na probe-tests (met o.a de volgorde ACDC) dat hij op de aanwezigheid van nieuwe elementtypen in liedjes reageerde, vooral als transities tussen verschillende elementtypen in zaten.

Een aantal van deze vogels werd vervolgens getraind en getest met de oude set liedjes (bestaande uit drie elementen), aangevuld met liedjes die uit vier elementen bestonden. Deze nieuwe liedjes (ABBA, AABB, BBAA, en ABAB) waren ook te onderscheiden met de 'eerste is het zelfde als het laatste element' regel. Echter, nu kon het onderscheid *niet* meer gemaakt worden door het letten op het aan- of afwezig zijn van herhalingen van elementen.

Twee van de vier getrainde zebravinken konden na de training het onderscheid maken, maar zij bleken allebei niet de 'eerste is zelfde als laatste element' regel te gebruiken. Na probe-tests bleek dat zij hoogstwaarschijnlijk de aan- of afwezigheid van gehele trainingsliedjes gebruikten om het onderscheid te maken. Hieruit bleek nogmaals het belang van het doen van probe-tests om meer te weten te komen *hoe* ze precies het onderscheid maken in dit soort experimenten. Bovendien lieten we zien dat er diversiteit kan zijn in welke regels zebravinken leren om het onderscheid te maken, ook al is de basis van de training hetzelfde.

Discussie

Na het doen van een zebravinkenzanganalyse met zang uit 13 populaties verspreid over drie continenten is gebleken dat alle elementtypen in alle populaties voorkomen, maar dat de relatieve verdeling verschilt tussen de populaties. Wat betreft natuurlijke fonologische syntax is er geen sprake van duidelijke syntactische patronen in natuurlijke zebravinkenzang op soorts- en continentniveau (wel in beperkte mate in een aantal populaties). Dit is verrassend, aangezien op individuniveau een motief een relatief stereotype, vaste volgorde heeft.

Wat betreft het waarnemen van grammaticale patronen of regels hebben we aangetoond dat zebravinken onderscheid kunnen maken tussen (artificiële) zebravinkenzangstimuli waarin de volgorde van de elementen verschilt. Een klein aantal zebravinken was zelfs in staat om wat ze geleerd hadden, te generaliseren naar nieuwe elementtypen, een belangrijke eigenschap van menselijke taal.

In eerste instantie klinkt dit misschien tegengesteld, maar het is mogelijk dat deze mechanismen om patronen/regels te detecteren in zang meer algemene cognitieve mechanismen zijn, en niet beperkt tot vocale productie en perceptie. Ze kunnen ook betrokken zijn bij ander gedrag dan zangperceptie en –productie, zoals baltsgedrag, aantallen/hoeveelheden inschatten, sociale verhoudingen, navigatie en fourageren. Ook bij mensen is aangetoond dat dit soort taken uit te voeren is met visuele stimuli en tonen, wat suggereert dat ook bij mensen deze mechanismen meer algemeen dan specifiek voor taal zijn.

Wat onze experimenten ook laten zien is het belang van het doen van probe-tests om te achterhalen wat de meest waarschijnlijke regel was waarmee het onderscheid gemaakt werd. Er bleek een relatief hoge mate van variatie tussen de individuele vogels in welke regels ze geleerd hadden om het onderscheid te maken, ook al waren ze getraind met stimuli met dezelfde elementvolgorde. In het verleden (en ook in onze eigen experimenten) is onderschat hoe moeilijk het is om experimenten te ontwerpen die maar met een enkel type regel opgelost kunnen worden.

In aanvulling op de variatie in regels die gebruikt kunnen worden om een taak in een experiment ‘op te lossen’, bleek ook dat de zebravinken waar mogelijk, minder complexe regels gebruikten dan die beoogd waren in de experimenten. Vanuit het oogpunt van *cognitieve efficiëntie* is dit ook te verklaren: waarom een relatief complexe regel leren en toepassen als een simpelere ook volstaat om het onderscheid mee te maken? Ook in dergelijke experimenten bij mensen is dit gevonden. Als een subject (dier of mens) geen complexe vorm van het leren van regels/patronen laat zien, betekent dit niet dat het/hij/zij niet in staat is om deze te leren, maar kan dat ook komen omdat die complexe regel niet nodig was om het onderscheid toch te kunnen maken. Bij het ontwerpen van toekomstige experimenten (in dier *en* mens) dient men nog meer beducht te zijn op deze *cognitieve efficiëntie* factor.

Het is mogelijk dat onze resultaten verband houden met de gekozen testmethode. Wij hebben de Go/No-Go methode gebruikt, waarin de zebravinken gedwongen werden om te laten zien dat ze verschillen hoorden in de stimuli. Er zijn ook andere methoden, waarin meer spontaan gedrag gebruikt wordt. Het voordeel van deze methode is dat er geen training in een Skinnerbox voor nodig is. Echter het is dan mogelijk dat, ook al kan het dier het verschil waarnemen, er geen ecologisch relevante noodzaak was om dat te laten zien in het gedrag. Mogelijk aanwezige competentie voor complex leergedrag kan dan niet aangetoond worden. We hebben wel geprobeerd een dergelijke methode voor zebravinken te ontwerpen, maar dit is tot nu toe zonder succes.

Conclusie

In dit proefschrift heb ik aangetoond dat er in natuurlijke zebravinkenzang wereldwijd elf verschillende elementtypen te onderscheiden zijn. Deze elf elementtypen komen in wisselende onderlinge verhoudingen voor tussen de verschillende populaties. Wat betreft syntax is op soortsniveau relatief weinig structuur aanwezig in zebravinkenzang, in tegenstelling tot de relatief stereotype volgorde van elementen op individuniveau. Wel is er op populatieniveau sprake van syntactische structuur bij 8 van de 13 populaties.

Wat betreft de perceptie van syntactische patronen bleken zebravinken verschillende volgorden van elementen in artificiële zebravinkenzang wel degelijk te kunnen onderscheiden, hetzij in beperkte mate. Een klein deel van de geteste zebravinken kon dit onafhankelijk van elementtype, een eigenschap die belangrijk is in menselijke taal. Dit lijkt in eerste instantie tegenstrijdig met het feit dat in zebravinkenzang op soortsniveau weinig syntactische patronen gevonden zijn. Dat zou kunnen komen omdat dit soort mechanismen van patroonherkenning meer algemene cognitieve vaardigheden zijn, iets wat eerder al gesuggereerd is na soortgelijke studies in mensen.

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Curriculum Vitae

1980	Born on the 29th of November in Alphen aan den Rijn, the Netherlands
1993-1999	VWO, Groene Hart Lyceum, Alphen aan den Rijn
1999-2006	MSc Biology, Institute of Biology Leiden (IBL), Leiden University (UL), *cum laude* Research project on learning-dependent song generalization in zebra finches (*Taeniopygia guttata*), supervised by Machteld Verzijden and Carel ten Cate (Behavioural Biology, IBL, UL)
2002-2006	BSc Psychology, Faculty of Social Sciences, UL, *cum laude* Honours Research Project on the influence of competition on the monitoring of internal and external feedback (with EEG), supervised by Katrien van Meel (Developmental and Educational Psychology, FSW, UL)
2008-2010	Party leader (one year) and council member for the Young Researchers (two years) in the University Council, the highest body of employee and student representation at Leiden University.
2006-2012	PhD project at Behavioural Biology, IBL, UL Title: On the production and perception of syntactical regularities in zebra finches: Experimenting with ABBA, ACDC and others, supervised by Carel ten Cate (Behavioural Biology, IBL, Leiden University). During this project I supervised several BSc and MSc students, gave multiple lectures and chaired discussion groups during the Behavioural Biology (IBL) and the Language and Cognition courses (Leiden University Centre for Linguistics, LUCL). I attended and presented posters and/or talks at several conferences: IBAC 2007 in Pavia, Italy; NVG 2007-2010 in Dalfsen, the Netherlands; VCiBM 2008 in St. Andrews, England; IEC 2009 in Rennes, France and Evolang 2010 in Utrecht, the Netherlands.

Curriculum vitae

I received funding from LUF to visit Duke University (North Carolina, USA) to collaborate with Rob Lachlan and Sita ter Haar on the zebra finch song database in Luscinia (Chapter 2 of this thesis).

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