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The Netherlands

The flexible listener: exploring zebra finch sensitivity to spectral and temporal sound features

Ning, Z.

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Female and Male Zebra Finches Preferences for Normal and Modified songs

Chapter



Zhi-Yuan Ning · Henkjan Honing · Peter Snelderwaard · Carel ten Cate

This chapter is prepared for submission

ABSTRACT

The zebra finch is an established model species for song preference research in songbirds. In this study, a 4-way choice operant paradigm (the ‘carrousel’ cage) was used to measure the spontaneous preference of adult birds of both sexes for song stimuli of four different categories: a conspecific song, a duration-stretched version and a vocoded version of the conspecific song, and a heterospecific great tit song. An earlier operant song discrimination experiment showed that both male and female zebra finches treated duration stretched and vocoded song versions as being highly different from a normal song. The current results show that female song preference for normal and duration-stretched conspecific songs was stronger than male preference. Females also exhibited a gradient of preferences: they preferred the normal and the duration-stretched song over the vocoded version of the conspecific song, but the vocoded conspecific songs were still preferred over the heterospecific great tit songs. Males preferred duration-stretched conspecific songs over heterospecific songs, but otherwise did not differentiate among the stimuli. Overall, our results demonstrate that results about the importance of particular song features as obtained in the context of an operant discrimination task and of a preference test paradigm can differ. This shows that combining different approaches and testing paradigms may help to better understand the significance of various acoustical parameters in relation to the ecological functions and evolutionary processes of birdsong as relevant to both sexes.

INTRODUCTION

The study of song preferences and song recognition in songbirds is a crucial element for understanding their vocal communication. Song preferences may indicate the birds' preference for the singers and play a vital role in model selection for vocal learning during the juvenile phase, as well as in mate selection in adult songbirds (Fujii *et al.*, 2022). In the context of vocal learning, research addresses whether conspecific song models are preferred over heterospecific ones (e.g., Marler & Peters, 1988) and which song features guide such preferences (e.g., Soha & Marler, 2000). Studies of song preferences in the context of mate choice examine which features make songs attractive (e.g., Vallet & Kruetzer, 1995; Gentner & Hulse, 2000) and whether these indicate specific qualities in the singers (e.g., Suthers *et al.* 2012; Wang *et al.*, 2019). Preferences may also be shown when birds can choose between familiar conspecific songs, e.g., those of fathers or individuals of the same population, and unfamiliar ones (e.g., Fujii & Okanoya, 2022). Additionally, songbirds of both sexes often recognize the songs of mates or neighbours (e.g., Stoddard *et al.*, 1990; Gentner *et al.*, 2000). Song preferences and song recognition can thus be expressed in different contexts and the song features on which responses to particular songs are based will vary according to the context. This ability implies the presence of an auditory perceptual system that is capable of identifying, selecting, and using different vocal features in different contexts: choice of a song model, selecting a mate, or recognizing a neighbour may all be based on different song features. This makes the study of auditory perception and the cognitive processes involved in song preferences and song recognition an important area of research.

The zebra finch (*Taeniopygia guttata*) has become an established model species for song preference research in songbirds. Both male and female zebra finches produce different types of calls, but only males produce songs, which consist of a series of repetitions of an individual-specific motif composed of three to eight different syllables (Zann, 1996). Song preferences in zebra finches have been extensively studied using various experimental paradigms, mostly either operant conditioning or phonotaxis experiments. The operant paradigm involves training a subject bird to perform a specific response, such as pecking a key or hopping on a perch, in order to elicit song playback as a reward. The number of responses is then used as an indicator of a song preference. This paradigm is used extensively in song preference research, mostly in studies that offered bimodal choices (e.g., Braaten & Reynolds, 1999;

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Neubauer, 1999; Houx & ten Cate 1999; Riebel, 2000; Riebel *et al.*, 2002; Riebel & Smallegange, 2003; Leadbeater *et al.*, 2005; Spencer *et al.*, 2005; Holveck & Riebel, 2007; Holveck & Riebel, 2010; Rodríguez-Saltos *et al.*, 2023; also see Riebel 2009 for a review) and occasionally also in studies that offered multiple choices (e.g., Ritschard *et al.*, 2010; Wei *et al.*, 2022). The phonotaxis paradigm involves presenting a subject bird with two or more different songs from speakers (or stimulus individuals) located on different sides of an experimental cage (Houtman, 1992; Neubauer, 1999; Adkins-Regan & Krakauer, 2000; Tomaszycki & Adkins-Regan, 2005; ter Haar *et al.*, 2014; Chen *et al.*, 2017; Fujii *et al.*, 2021; Mol *et al.*, 2021). The bird's approach to one or another speaker is taken as an indication of its preference for the corresponding song. This method is based on the natural behaviour of zebra finches to approach preferred songs (e.g., Loning *et al.*, 2023). In the operant conditioning paradigm, birds must work spontaneously to trigger a song playback, so operant conditioning is more closely related to the motivation to hear a preferred song than the phonotaxis paradigm for detecting song preferences.

The use of different paradigms and stimuli/sounds has allowed for a comprehensive investigation into zebra finch song preferences and has shed light on the features underlying these preferences and the role of experience in shaping them. This demonstrated, among others, that zebra finches of both sexes preferred conspecific song over either the songs of cross-fostered Bengalese finches (*Lonchura striata* var. *domestic*) (Clayton & Pröve, 1989; Campbell & Hauber, 2009), or European starling (*Sturnus vulgaris*) (Braaten & Reynolds, 1999), and that young adult females preferred conspecific to canary (*Serinus canaria*) song in the two-choice preference test paradigm (Lauay *et al.*, 2004). Among conspecific songs of zebra finches some types of songs are preferred over others, such as longer songs over shorter ones (e.g., Clayton & Pröve, 1989; Neubauer, 1999), more complex songs over less complex ones (e.g., Woodgate *et al.* 2011), faster songs over slower ones (e.g., Chen *et al.*, 2017), and songs with better-defined harmonic elements over those with noisier elements (Tomaszycki & Adkins-Regan, 2005). Additionally, both males and females prefer the songs of their father or other model song over unfamiliar songs (e.g., Houx & ten Cate, 1999; Riebel *et al.*, 2002) and over the songs of unfamiliar brothers (i.e., same parents but different clutch) (Riebel & Smallegange, 2003), and juvenile male zebra finches prefer the songs of their father over the songs of another familiar adult male during the auditory phase of song learning (Rodríguez-Saltos *et al.*, 2023) and song stimuli consisting of more common zebra finch song elements over stimuli consisting of less common elements (ter Haar *et al.*, 2014). However, while the

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studies mentioned above indicate preferences for certain types of songs, they don't necessarily provide much insight into the nature of the song features on which these are based or on the perceptual and cognitive abilities that zebra finches have at their disposal to detect differences between songs. For instance, the finding that zebra finches prefer conspecific songs over heterospecific ones or familiar ones over unfamiliar ones shows that they can distinguish these, but it does not reveal on which acoustic parameters the discrimination is based. Also, the absence of a preference among various songs does not imply that zebra finches are insensitive to the acoustic differences between these songs. To address such issues, a different approach is required examining which features birds use, or are capable of using, for discriminating between songs or other auditory stimuli. This is done in experiments using operant discrimination paradigms such as a Go/No-go or Go-left/Go-right training, in which birds are trained to obtain a food or water reward for responding to one type of sound and not, or in a different way to another sound. Errors in responding can be followed by a signal such as a brief period of darkness or do not result in a reward. To address which features the birds use to discriminate between the training sounds they are given test sounds that systematically alter specific sound parameters and examine the impact on the ability to still recognize or distinguish the sounds. These discrimination experiments have explored the type of acoustic parameters to which zebra finches are able to attend in discriminating songs and other sounds, such as pitch, intensity (Nagel *et al.*, 2010), spectro-temporal structure of elements (Dooling *et al.*, 2002; Lohr *et al.*, 2006; Vernaleo *et al.*, 2010; Vernaleo & Dooling, 2011; Lawson *et al.*, 2018; Prior *et al.*, 2018; Fishbein *et al.* 2021), spectral envelope (Burgering *et al.*, 2019), element sequences (Lawson *et al.*, 2018; Geberzahn & Derégnaucourt, 2020; Ning *et al.*, 2023) and duration of song (see Chapter 2).

It is important to note that operant discrimination experiments also have limitations in revealing the full extent of the cognitive processing involved. Specifically, while changes in a specific parameter may result in reduced recognition of a song, this does not reveal whether such a modified song is no longer recognized as a zebra finch song or is considered unattractive. As an example, our previous studies (Ning *et al.*, 2023; Ning *et al.*, submitted - Chapter 2, Chapter 4) showed that when song stimuli in an operant discrimination task were changed by vocoding – replacing a harmonic spectrum by noise in the same frequency range – they were treated as being highly different from the original training songs. This showed that the harmonic spectrum was a critical element for distinguishing the training songs. It may suggest that a vocoded song is no longer recognized as a zebra finch song at all and considered just as

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different from the normal song as heterospecific song or an arbitrary sound, but that need not be the case. A vocoded song version still contains some species-specific information, e.g., it maintains the temporal structure and frequency ranges of song syllables. This might still make such a stimulus interesting to zebra finches. Thus, while modifications made to training songs in the context of an operant discrimination task may result in song stimuli that are considered very different from the training songs it is an open question whether such manipulated stimuli still retain sufficient specificity to be recognized and potentially even be attractive to zebra finches. This can only be assessed by presenting such manipulated stimuli in a preference paradigm in which the preferences of zebra finches for normal songs and manipulated stimuli are compared.

In the current experiment we examine how the preference for non-manipulated songs (used as training stimuli in an earlier operant song discrimination study; see Ning *et al.*, submitted - Chapter 2) relates to the preference for some of the manipulated versions of these songs as well as to the preference for a heterospecific song. This allows for examining whether song modifications, that reduce the recognition of a familiar song in a discrimination task, also cause such stimuli to be less attractive in a preference test. Since zebra finches prefer conspecific songs over heterospecific ones (Clayton & Pröve, 1989; Braaten & Reynolds, 1999; Lauay *et al.*, 2004; Campbell & Hauber, 2009), we expect less attractive song variants to be treated more similar to heterospecific songs than to normal ones. Our song discrimination study showed no sex difference into which features and how strongly males and females responded, but this does not imply that males and females have similar song preferences. We therefore examined the song preferences of adult birds of both sexes in an identical context and with the same stimuli and method – something in itself of interest as this is not often done.

We tested zebra finch preferences in a four-way preference set up, using an octagonal choice cage in a multiple, interactive choice situation, or “carrousel” for short. This operant preference paradigm has been designed and successfully used in our laboratory’s previous preference research including preference tests on imprinting, courtship, and birdsong (ten Cate *et al.*, 2006; Holveck *et al.*, 2011; Wei *et al.*, 2022). Song preference tests in this set up demonstrated that zebra finches will voluntarily hop on an operant perch if this results in hearing a conspecific song, with this song exposure acting as a reinforcer for this behaviour (Wei *et al.*, 2022). The individual preferences were measured by the number of times the bird triggered playbacks of four different stimuli: a normal zebra finch song, its duration-stretched

version, a vocoded version, and a heterospecific song. Zebra finches considered both the duration stretched, and the vocoded version of normal songs as being significantly different from the normal training songs in an operant discrimination task (Ning *et al.*, submitted).

METHODS

Subjects

Forty-five zebra finches (23 males and 22 females) were used in this experiment. They were tested at the age of 298 ± 50 days post-hatching (dph), (age males: $M = 318$, $SD = 60$, age females: $M = 281$, $SD = 31$). All birds originated from the in-house breeding colony at Leiden University. Before the experiment, the birds lived in single-sex groups of about 15 to 30 individuals in aviaries ($2m \times 2m \times 1.5m$), in which food and water were available ad libitum. The housing rooms were kept at $20\text{--}22^\circ\text{C}$ and 40–60% humidity and illuminated with artificial lights (Philips Master TL5 HO 49W/830) from 07:00–20:30 (13.5h light : 10.5h dark) with a 15 min twilight phase with the light fading in and out at the beginning and the end of each day. A week before the preference test, birds were caught and transferred from the aviary to standard laboratory cages (two birds of equal sex in one cage) in order to acclimatize (cage size length $\times$ width $\times$ height: $80 \times 40 \times 40$ cm) and reduce stress from catching in aviary.

Experimental setup

The birds were tested individually in a 4-way preference choice cage (carrousel cage) which was placed on a table (height: 75 cm) within a sound attenuated chamber (height: 250 cm, width $\times$ length irregular quadrilateral: $335 \times 280 \times 290 \times 300$ cm). The experimental setup consisted of a central octagon cage (height: 35 cm, diameter: 70 cm) containing 8 perches, of which 4 were sound perches (sensors), triggering playback of a sound when the bird hopped on them, and 4 were silent perches, not resulting in a sound being played. The cage had a wire-mesh covering (height: 35 cm, diameter: 70 cm) on its top and 8 wire mesh openings, one on each side closed off with white plastic partitions. Four sides had a loudspeaker (CB4500, Blaupunkt, Hildesheim, Germany) on the outside, broadcasting through a round opening (diameter = 3 cm) in the middle of the plastic partition (see Fig. 1). The birds triggered song playbacks by landing on small cross-shaped perches in front of the loudspeaker openings, which activated a microswitch connected to a computer outside the experimental room. The computer controlled the playbacks and recorded the moment the bird landed and departed

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(Dell OptiPlex 3010 with RADEON HD soundcard and custom-written software in Visual Basic 6, by P.C. Snelderwaard). Hopping on a sound perch triggered a single playback sound, regardless of how long the bird stayed on a perch. However, if the bird left the perch before the end of the song playback and hopped to another sound perch, the previous playback was stopped and a new playback associated with the new perch was triggered. Prior to the experiment, playback levels on each perch were set to the maximum level of 68 dBA (re: 20 μ Pa) using a VOLTCRAFT SL-100 sound meter with the sound level set to high and A-weighting. A one-way mirror window in the door allowed observation of the room from outside but did not allow visibility of the birds everywhere in the carrousel. Therefore, a camera (Logitech HD 1080p, Lausanne, Switzerland) was mounted 1.5 meters above the carrousel and connected to the computer (software: Webcam7, v. 70.9.9.41) controlling the setup, allowing monitoring of individuals' behaviour during trials from outside the experimental chamber.

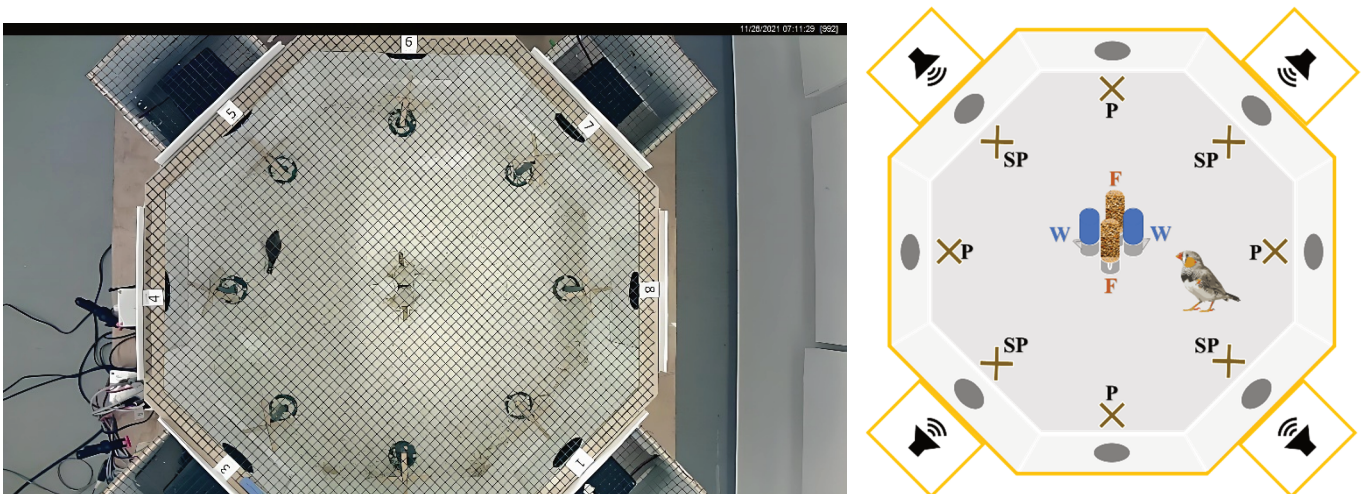


Figure 1. Experimental apparatus (Carrousel cage) used for the preference test. a) Photograph top view of the apparatus, and b) Schematic representation of the testing apparatus: Four **loudspeakers** are positioned symmetrically behind the plastic partitions on the diagonal side of the octagonal cage; eight grey ellipses represent the **round openings** through half of which the sounds of loudspeakers were broadcasted. Within the cage, there are eight cross-shape perches for the bird to sit on, with four of them being **sound perches** (SP) and the other four being **silent perches** (P). Two tubes with ad libitum **water** (W) and two tubes with ad libitum **food** (F) are placed in the middle of the floor panel. Landing on a sound perch triggered a single playback of the stimulus associated with it while landing on a silent perch resulted in no playback. The stimuli associated with the four sound perches were rotated one position clockwise per block, with each block lasting for two and a half hours.

Stimuli

Four types of stimuli were used in probing the preferences of the birds.

Normal zebra finch song

These songs consisted of motifs from the songs that also had been used as training stimuli for the Go-Left/Go-Right operant task in chapter 2. They consisted of a natural motif repeated several times without introduction notes. These motifs were extracted from representative recordings of adult males whose vocalizations had not been heard before by the experimental birds in this preference test. The same motif was repeated 3 to 6 times (resulting in a duration of 3.5 - 4.5 seconds). When played, the motifs within a song were normalized in root-mean-square (RMS) amplitude such that the average intensity was the same but the range of variation in volume recorded at the microphone was preserved. All zebra finch songs were filtered to a bandwidth between 380Hz and 15kHz (Fig. 2a). The songs were cut, synthesized, and filtered using Praat (version 6.0.54). The amplitude of each song was adjusted by using the “Normalize” feature in Audacity (version 2.3.0).

Duration-stretched zebra finch song

For the Duration-stretched manipulation, the duration of the whole song was stretched proportionally without any change in the frequency domain (Fig. 2c). The “change duration” script of Praat Vocal Toolkit was applied to obtain the 50% Duration-stretched versions (see the “duration +50%” stimuli version of test series 1 in chapter 2).

Vocoded zebra finch song

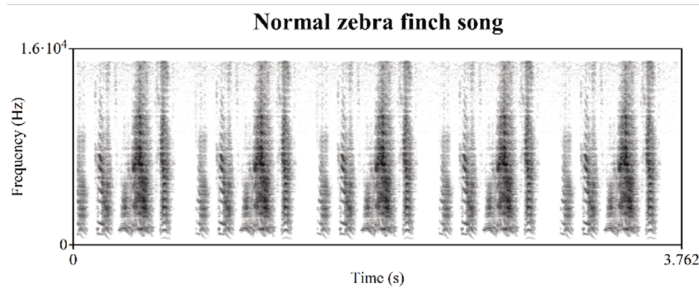
This song modification maintains the spectral and temporal envelopes of the elements within the motif, but averages the energy within specific frequency bands of the song elements, thus removing any harmonic structure (Fig. 2b). The vocoded version of songs was constructed by the Matt Winn’s Praat vocoded script (http://www.mattwinn.com/praat/vocode_all_selected_40.txt) which maintains the temporal envelope (see the Vocoded stimuli of test series 2 in chapter 2). This script was set to divide cut-off frequency bandwidths equally for 15 bands contiguous with smooth transitions (1000Hz bandwidth for each noise-vocoded band).

Heterospecific song

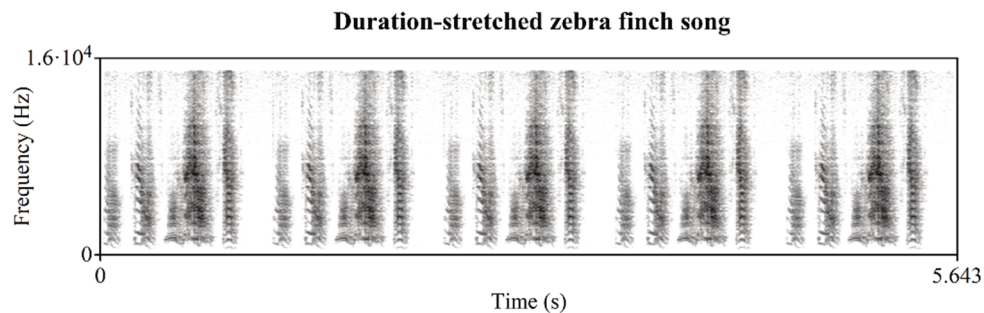
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The heterospecific songs were selected from the field recordings of natural great tit (*Parus major*) songs. Great tit songs consist of repetitions of fixed syllables that deviate from the spectro-temporal structures of zebra finch conspecific songs. The total duration of a heterospecific song was selected to be similar to the duration of the zebra finch song in the stimuli set tested for a same individual (Fig. 2d). To obtain clear sounds from those field recording with which the environmental background noises were along, the “reduce noise” function of Praat Vocal Toolkit was applied to eliminate the background noise, and the denoised great tit songs were then high pass filtered at 2000 Hz by using Praat and were normalized as the same RMS amplitude as the zebra finch songs by using Audacity.

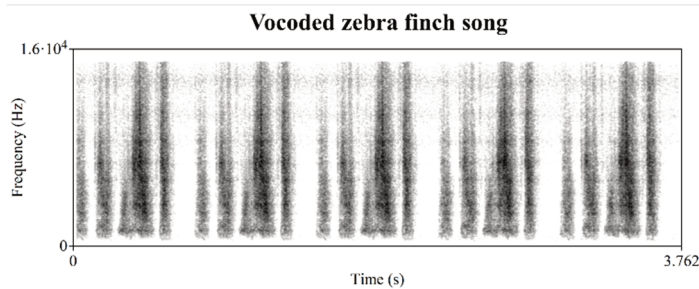
a



b



c



d

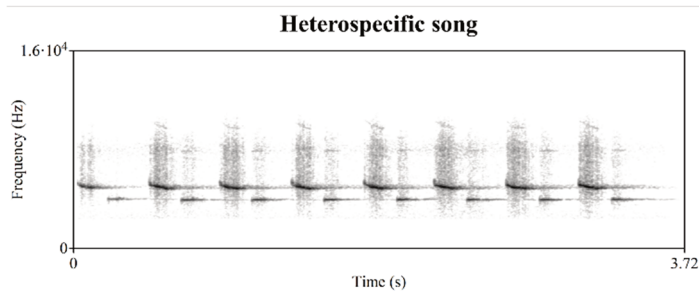


Figure 2. Spectrogram examples of four different stimuli used for the preference test. a - the original version of a **Normal zebra finch song**. The **Duration-stretched** version (b) stretched the normal song by 50%, and the **Vocoded** version (c) was produced by using the Matt Winn's Praat vocoded script. The **Heterospecific song** (d) was the song of a great tit of similar song duration but different in both spectral and temporal structure of elements compared to the original version of the zebra finch song.

Procedure

The experimental procedure consisted of two sessions: acclimatization and preference testing (Fig. 3). In the acclimatization session, each bird was placed into the carousel at 17:00 the day before the preference testing and given ten hours overnight to acclimate. A five-seconds ambient sound recording of their aviary was triggered if the bird hopped on one of the sound perches before the light was turned off at 20:30. In this way, the birds were expected to learn that sound playback was associated with some of the perches before being transferred to the real testing session. The preference test lasted from 7:00 to 17:00 on the second day, and each of the four test stimuli was assigned to a specific sound perch. The stimuli were randomly assigned to the positions of sound perches, and the stimulus category assigned to a particular sound perch differed among birds. For each stimulus category, there were multiple songs, and each variant was only used for one or a few birds. These sound perch assignments were rotated one position clockwise every two and half hours, which constituted one "block", until each stimulus had been presented at each perch position once. Thus, the entire preference test was consisting of four blocks. Like the experiment by Wei *et al.* (2022), an additional loudspeaker (JBL Clip2) was placed on the floor directly under the carousel broadcasting a continuous recording of ambient sound from the bird aviaries (45 dBA sound peak pressure level measured at the height of the perches inside the carousel) to maintain the acclimation half hour before and during the session of preference testing. Ad libitum access to food and water was available throughout experiment, the birds were returned to their home aviaries after the testing was completed at 17:00.

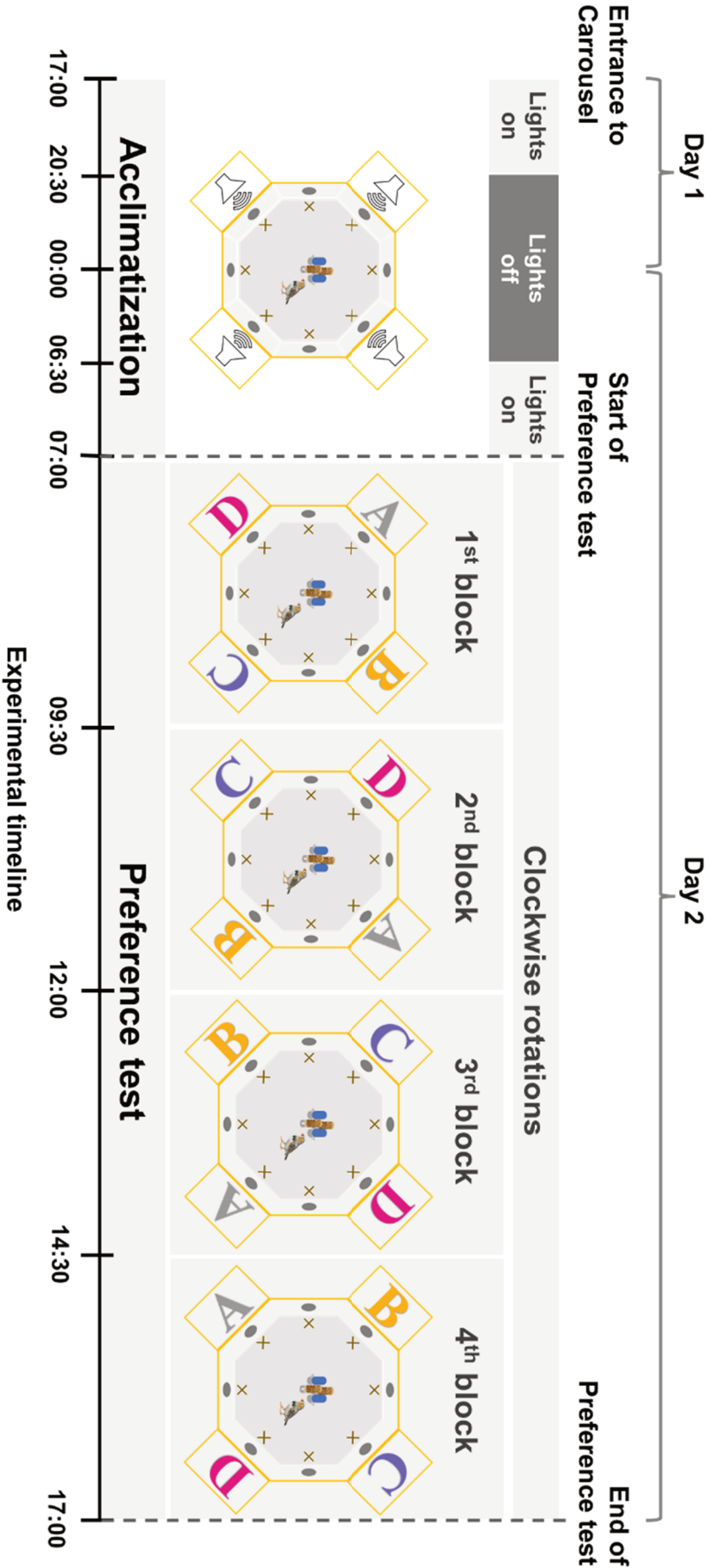


Figure 3. Experimental procedure. In the **Acclimatization session**, a bird was moved in the setup in the afternoon of the 1st day to acclimate the carousel cage and the operant perches, and then to stay overnight. The **Preference test** session started in the morning of the 2nd day and lasted for ten hours; the stimuli (the labels A/B/C/D marked with different colors represent the different test sounds) associated with the four sound perches were rotated one position clockwise per each 2.5h block.

Analysis

The number of sound perch visits were chosen as a variable indicating a bird's preference toward a particular stimulus. For each bird, the number of visits on each sound perch was extracted from the data files registered individually by the logger software (original file format: text, transferred and saved into excel spreadsheets, v. 16.33). As reliable preferences can only be assessed in birds that visited all four perches giving them exposure to all the stimuli, our data analysis included only the counts of perch visits after a bird had hopped on all sound perches and had triggered all stimuli at least once (during the preference test session). Also, all stimuli had to have been visited at least once before 12:00 (at least underwent two full blocks) on day 2, and birds had to remain active and emit at least a total of 120 perch visits during the preference test session (average 30 perch visits per stimulus) to be included in the analysis. This resulted in 35 out of 45 birds reaching the criteria (with on average 851 ± 517 perch visits, range: 133–1942), which includes 16 males (with 670 ± 409 perch visits, range: 133–1509) and 19 females (with 1003 ± 547 perch visits, range: 156–1942).

A Generalized Linear Mixed-effects Model (GLMM) was used to assess the relative preferences that birds show among the 4 stimuli. This model analysis was conducted in Rstudio (R Core Team, 2016). We used the “Number of visits” for the various stimuli as response variable in GLMM. We used “Test_Stimulus” (Four versions of test stimuli), “Sex” (Male/Female), and the interaction between these two as covariates in the model with “Bird_ID” and “Stimulus_ID” as the random factors and a Poisson error structure of the “Number of visits”. To assess whether any explanatory variables in a model were significant, we used Wald Chi-Squared tests to evaluate the fixed effects of the model (using the function `car::Anova`, R package `car`; Fox & Weisberg, 2019). A post-hoc analysis is performed on the final model with FDR correction (using the `emmeans` function, R package `lsmeans`; Lenth, 2016).

Ethics Statement

All animal housing, care, and use was performed with the permission of the Leiden Committee for Animal Experimentation (AVD number 1060020197507). None of 45 birds had any experience with this experimental setup or the stimuli preceding the experiment. Before being

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transferred to the setup, each experimental bird was co-housed with one other bird and underwent a physical examination. A brief removal from the social groups, which lasted no more than 24 hours, and behavioural observations in an open choice arena were considered non-procedural under the Experiments on Animals Act (Wod, 2014), which is the applicable legislation in the Netherlands, in accordance with the European guidelines (EU directive no. 2010/63/EU) for the protection of animals used for scientific purposes. The health and welfare of these birds was monitored by licensed and skilled personnel during the experiment, and all procedures were reviewed and monitored by the official Animal Welfare Body responsible for monitoring and implementing legal requirements. When showing signs of stress, deviating behaviour or illness a bird would be removed from the setup.

RESULTS

The ANOVA Type III showed that the number of perch visits differed significantly among the different test stimuli, and a trend towards a difference between males and females. Thus, the two factors “Test_Stimulus” and “Sex” as well as their interaction effects for the response variable “Number of visits” were selected as fixed factors for model analysis (see Table 1)

Table 1 ANOVA (Type III Wald chi square tests) table for selected GLMM

Variable	Chisq.	Df	Pr(>Chisq.)
MODEL: Number of visits			
Test_Stimulus	82.0014	3	< 2e-16 ***
Sex	3.4825	1	0.06202 .
Test_Stimulus:Sex	8.3438	3	0.03942 *

Note: GLMM with a Poisson distribution. All variables shown here were the variables of our interest and were thus used as fixed factors for the GLMM. “Bird_ID” and “Stimulus_ID” were included as two random intercepts.

• $0.05 < p \leq 0.10$, * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$.

For the females, the perch visits to the Normal zebra finch song and its Duration-stretched version were significantly higher than to its Vocoded version and the Heterospecific song (both $p < 0.001$). In addition, the females visited the Vocoded zebra finch songs more often than the Heterospecific songs ($p < 0.05$). For the males, the perch visits to the Duration-stretched zebra finch song were significantly higher than to the Heterospecific song ($p < 0.01$), but there were no significant differences among the other stimuli. Overall females were more active than males and the pairwise comparisons per stimulus between males and females by the Post hoc Tukey's HSD tests showed a clear trend to a difference between two sexes'

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towards more perch visits by females to both the Normal zebra finch song and its Duration-stretched version (both $p = 0.06$) (Fig. 4).

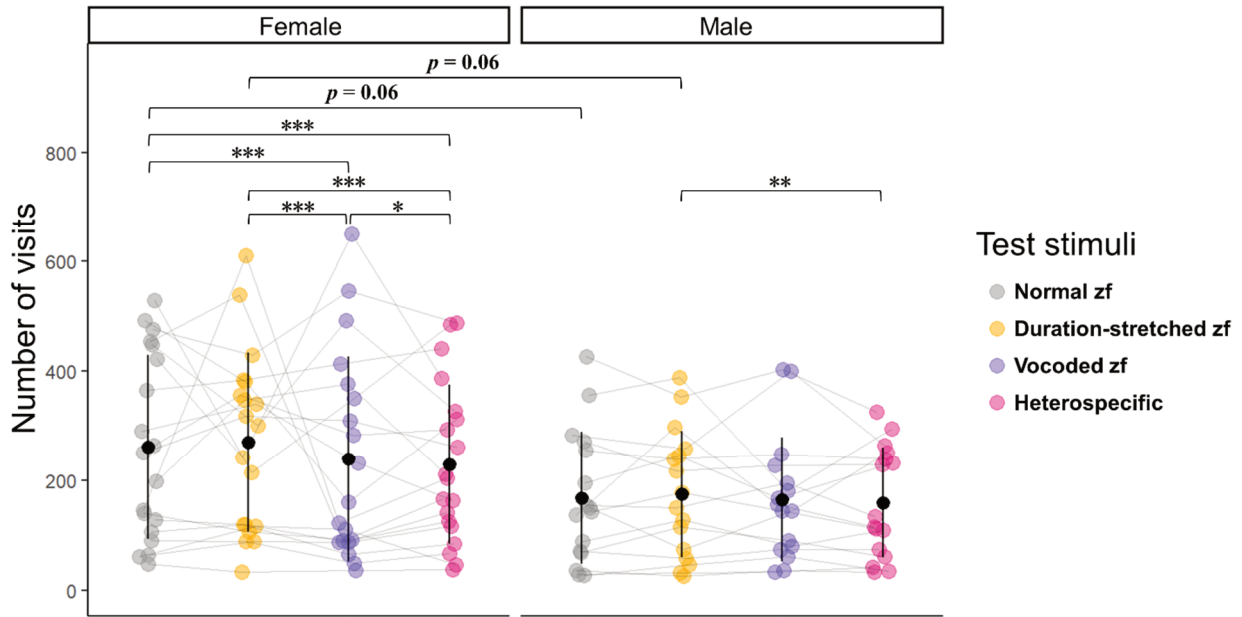


Figure 4. Number of sound perch visits for each sex group toward the test stimuli. *** refers to a significant difference of $p \leq 0.001$, ** refers to a significant difference of $0.001 < p \leq 0.01$, and * refers to a significant difference of $0.01 < p \leq 0.05$, for non-indicated comparisons p value is > 0.1 . Error bars along with a black dot indicate Mean $\pm$ SD in perch visits to each test stimulus.

DISCUSSION

In this study, we examined the spontaneous preference of adult birds of both sexes for four different song stimuli. Both adult male and female zebra finches display a preference for normal or duration stretched conspecific songs over heterospecific ones. This finding is in line with other studies (Clayton & Pröve, 1989; Braaten & Reynolds, 1999; Lauay *et al.*, 2004; Campbell & Hauber, 2009), confirming that song plays an important role in species/subspecies recognition. However, while other studies found no sexual differences in zebra finches' preference of conspecific over heterospecific songs (e.g., Braaten & Reynolds, 1999), we observed a strong trend towards a sex difference, with females showing stronger preferences than males for normal zebra finch songs and duration-stretched ones. The observed sex difference most likely reflects that sexual attraction plays a key role in female zebra finches' conspecific song preference, while the motivation of male zebra finches to respond to

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conspecific song may serve other social functions (e.g., Loning *et al.*, 2023) different from the mate-choice driven motivation of females.

Our results further show that female zebra finches exhibited a gradient of preferences: they prefer conspecific songs that had not been manipulated in the spectral domain (nonmanipulated zebra finch song and duration-stretched zebra finch song), over the vocoded version of conspecific song, but vocoded song is preferred over the heterospecific great tit song. In contrast, the males don't differentiate among the stimuli, apart from a preference for the duration-stretched zebra finch song over heterospecific songs. The finding that males respond less than females and that their responses hardly differ towards the song variants makes it hard to conclude that males show a preference for a particular song variant over others. Overall, the results thus show that female zebra finch song preferences are more outspoken than male preferences, as well as more selective.

In our song discrimination study (see Ning *et al.*, submitted - Chapter 2), we showed that both duration-stretched and vocoded song versions strongly reduced song recognition. However, in the current experiment, both manipulations differ substantially in their impact on the preference. Duration-stretched songs are at least as attractive as normal songs and preferred over vocoded ones by females and over heterospecific ones by males. Several earlier studies on song preferences showed that zebra finch females preferred longer or more complex songs (e.g., Clayton & Pröve, 1989; Neubauer, 1999), although this was not always the case (e.g., Holveck & Riebel, 2007; Riebel *et al.*, 2009). Therefore Riebel (2009) argued that the duration of song is not a good explanatory variable for female song preference. However, in those studies longer songs differed in element composition and number from shorter ones, while in the current study, the duration-stretched manipulation on zebra finch songs didn't change the complexity in frequency domain or the number of syllables within the original song but stretched song duration proportionally, thus slowing down song tempo. This manipulation did not affect the preference for the song. The same manipulation did however affect the recognition of a training song when it was used in an operant discrimination task, indicating that it is a feature to which zebra finches can and do attend, but they apparently don't do this in the context of a preference test.

Vocoded songs are less attractive to females than normal songs. Such a response may be comparable to the finding that normal songs with noisier elements were found to be less attractive than those with a better-defined harmonic spectrum (Tomaszycki & Adkins-Regan,

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2005). Nevertheless, vocoded songs are still preferred over heterospecific songs. Noise elements with acoustic energy more or less uniformly distributed over all frequencies without any spectral structure and resembling a band of white noise have been reported to be natural element types present in zebra finch songs (Zann, 1993; Lachlan *et al.*, 2016). Thus, although the noise-vocoded manipulation did result in substantial reduction of conspecific song recognition in both sexes in our discrimination studies (Chapter 2 & Chapter 4), natural songs modified in this way still retain sufficient specificity to be identified as a zebra finch song by female zebra finches. This may be based on the spectral envelope and temporal features (i.e., spectral contour) of the song syllables, which are still maintained after noise-vocoding. Nevertheless, it is clear that vocoded songs are less attractive than normal and duration stretched songs. So, while in our operant study both duration stretched and vocoded song versions were affecting song recognition, only vocoding had a negative impact on the preference.

To conclude, our study shows that modifying specific song features might have a different impact on song recognition than on song preference. Also, while no sex differences were observed in our operant song recognition study, they were present in the preference test. It demonstrates that results about the importance of particular song features as obtained in the context of an operant discrimination task and of a preference test paradigm can differ. Our results thus show that combining different approaches and testing paradigms may help to better understand the importance of various acoustical parameters in relation to the ecological functions and evolutionary processes of birdsong as relevant to males and females.

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