

Songs to remember

On the functions of song memorisation in
female zebra finches

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Abstract

Memorisation brings along costs, therefore certain benefits must outweigh these costs. Zebra finches are able to memorise songs, this takes place both early in life and throughout life. Males use memories of songs in order to develop an own song. However, female zebra finches do not sing, and therefore song memorisation in females must serve other purposes. We discussed the functions of song memorisation in female zebra finches based on current literature.

We explored the functions of song memorisation in recognition and female mate choice. It is shown that female zebra finches are able to recognise conspecifics, their own subspecies and their mate. Furthermore, song memorisation in female zebra finches could play a role in mate choice. Females learn to prefer songs of tutored males of their own subspecies by memorising songs. Besides, avoiding inbreeding and finding a local mate could be functions of song memorisation as well, but further research needs to clarify this.

Concluding, to some extent, recognition and mate choice seem to be functions of song memorisation. Why birdsong evolved remains a major question, although the functions of song memorisation could shed light on this problem.

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Introduction

The ability to learn is common throughout the animal kingdom (Kirkpatrick & Hall, 2005). Learning serves a role in adapting to local circumstances and requires the formation of memories. Memorisation brings along neural costs, it is thought to be associated with a neural process called long-term potentiation (LTP), which results in increased long-lasting synaptic strength (Purves et al., 2012). Songbirds are capable of memorising songs. This means the benefits of song memorisation exceed the costs of this ability.

Song memorisation, the storing of a specific song in a bird's memory, is needed for the development of songs. Another feature of birdsong is that it, just like human language, needs to be acquired. Both are forms of vocal learning, which is defined as "the ability to acquire vocalisations through imitation rather than instinct" (Jarvis, 2004, p. 749). Only a few groups of animals seem to possess this ability: primates (including humans), bats, cetaceans, parrots, hummingbirds and songbirds (Jarvis, 2004).

In songbirds not all memorised songs are used in vocal production learning, birds are capable of auditory learning as well: the capacity to make associations with sounds an animal has heard (Clayton, 1988; Jarvis, 2004, p. 749). This means songs are memorised, but that they are not used for the production of songs. Since song memorisation brings along costs, there must be certain benefits to outweigh these costs. Thus, auditory learning must serve certain functions.

The zebra finch (*Taeniopygia guttata*) is a non-human model organism for vocal learning and many researchers study this species. Although in most songbird species, including zebra finches, only the males sing, both male and female zebra finches are capable of auditory learning. Both sexes are able to memorise their tutor's song early in life and these memories persist to adulthood (Clayton, 1988; Holveck & Riebel, 2014). Furthermore, later in the life of zebra finches songs of conspecifics are added to the memory in both sexes as well (Miller, 1979; Stripling et al., 2003).

Male songbirds have extensively been investigated, although knowledge of female songbirds extends less far (Riebel, 2003a). The benefits of song memorisation may differ between males and females, since only males use song memorisation in the production of songs. Female zebra finches do not sing, and therefore song memorisation must serve other purposes in females. This thesis explores the possible functions of song memorisation in female

zebra finches by discussing the research question: *What are the functions of song memorisation in female zebra finches?*

Riebel (2003a) described that auditory learning can influence vocal perception in female songbirds in two ways: 1) recognition and 2) preference. She defines 'recognition' as playing a role in the identification of a conspecific or a potential mate, and 'preference' as affecting a choice between potential mates. In this thesis recognition and preference are discussed as possible functions of song memorisation in female zebra finches.

This thesis starts with a general chapter on vocalisations and memorisation in zebra finches. Next, the role of song memorisation in recognition and female mate choice are discussed in chapter 2 and 3, respectively. This thesis ends with a conclusion on the function of song memorisation in female zebra finches based on current literature on memorisation of birdsong.

Chapter 1 **Vocalisations and memorisation**

In this first chapter background information on vocalisations and memorisation is provided. Besides, it is discussed when song memorisation takes place, how song memorisation is tested and what acoustic features of songs are memorised.

1.1 Vocalisations

In general, two kinds of vocalisations are distinguished in zebra finches: calls and songs (Zann, 1996). In zebra finches, females only produce calls, while males produce both calls and songs. Broadly, male-male competition and mate attraction are distinguished as functions of song production (Catchpole & Slater, 1995). The zebra finch is a non-territorial species that lives in large flocks, and therefore male-male competition is thought to be less important than in territorial songbird species (Zann, 1996).

Male zebra finches sing only one particular song in their life. In addition to the innate template (Catchpole & Slater, 1995), they develop this song by learning from a tutor in a two-step process. First they memorise their tutor's song in the memorisation phase, followed by the sensorimotor phase that comprises vocal practice (Doupe & Kuhl, 1999; Bolhuis & Gahr, 2006). These two phases partly overlap and end at approximately 100 days post-hatching (Bolhuis & Gahr, 2006). After this age the zebra finch's song is 'crystallised', which means the song remains unchanged afterwards. A zebra finch's song resembles his tutor's song, though it also differs in some aspects. Consequently, each zebra finch sings a unique song (Zann, 1996).

Songs of zebra finches can be divided into directed and undirected songs (Zann, 1996). Directed songs are mostly sung in the presence of a female during the courtship dance and indicate a male's willingness to mate (Zann, 1996). The functions of producing undirected songs are less clear, since it is sung when a male is alone. Furthermore, directed and undirected songs differ in acoustic features. For instance, a directed song is sung faster compared to an undirected song (Zann, 1996).

1.2 Memorisation of songs

Zebra finches are capable of memorising songs of their tutor (Clayton, 1988; Holveck & Riebel, 2014) and of other conspecifics (Miller, 1979; Stripling et al., 2003). We distinguish two time spans of song memorisation, based on the age at which memorisation takes place. These are song memorisation early in life and throughout life, and these are discussed in the next sections.

1.2.1 Memorisation early in life

For both females and males, memorisation early in life takes place during a sensitive phase, this is “the developmental period where learning about a particular stimulus occurs without an obvious external reinforcer” (Riebel, 2003b, p. 79). In males, the peak of the sensitive phase is approximately from 35 days to 65 days post-hatching and this is probably comparable in females (Holveck & Riebel, 2014). As discussed earlier, males memorise their tutor’s song in order to develop an own song, and this takes place during the sensitive phase. If male birds are not exposed to songs during this phase, males will develop an abnormal song (Immelmann, 1969, as cited in Zann, 1996; Fehér et al., 2009).

Females are able to memorise more than one song during the sensitive phase (Holveck & Riebel, 2014). It is not yet clear from whom females memorise these songs under natural circumstances. We can hypothesise one of these memorised songs is their father’s, since the young females are probably frequently exposed to this song when they are in and near the nest (Immelmann, 1969, as cited in Zann, 1996). Tutor song memorisation does not play a role in song production learning in females, since females do not sing.

1.2.2 Memorisation throughout life

Besides tutor song memorisation in the sensitive phase, memorisation of conspecifics’ songs also takes place later in the life of zebra finches of both sexes (Miller, 1979; Stripling et al., 2003). In the latter type of memorisation there is no sensitive phase, songs can be added to the memory anytime.

1.3 Testing song memory

In investigating female mating preference either live males or song playbacks are used (reviewed in Holveck & Riebel, 2006). In a live male test a female is able to choose between two (or more) males. Which male is preferred is determined by observing the behaviour of the female.

However, if preference for a male’s song needs to be investigated, preference tests in which songs are played back could be used. There are two types of song playback preference tests: phonotaxis tests and operant tests (Holveck & Riebel, 2006). In phonotaxis tests two (or more) different songs are played back to a female. By scoring and analysing the behaviour of this female in response to the songs, female preference can be determined. In this kind of tests the female is not able to interact with the stimuli. In operant tests, a female is taught to peck a

button that triggers song playback. By measuring what songs a female plays back more often female song preference is determined. A disadvantage of operant tests is that learning a bird to peck a button takes a long time and is unnatural for birds.

These three types of preference experiments are used to investigate whether a female reacts different to a stimulus compared to another. In case a song is memorised, we would expect that a bird is able to recognise this memorised song. This would show in a different behavioural response to the memorised song in comparison to other songs. In operant tests, we would expect a difference in button pecking. Therefore, these two types of playback experiments are used to test song memorisation.

In playback experiments, birds could also perform different behaviour during one song compared to another because one song is more attractive and not because the bird recognises this song. To correct for this, behaviour in response to several novel songs are used to compare the behaviour of the (potentially) memorised song to, and multiple test birds are used.

1.4 What acoustic features are memorised?

Before the functions of memorisation of songs are discussed, a short overview is provided on the mechanism of song memorisation. A memory is formed based on a heard song, and later this memory plays a role in recognition. What does this memory consist of, what acoustic features of a song are memorised?

First some terminology of birdsong structure is explained. A song bout consists out of one or more motifs, and a motif is a sequence of syllables in a specific order (Zann, 1996). The smallest units of syllables are elements, and some elements resemble the tutor's elements more closely than others. When 'song memorisation' in this thesis is mentioned, 'song' refers to one motif.

We describe the structure of a song as consisting of three different facets: pitch, amplitude and duration. The pitch refers to the frequency of a sound wave in a song; a higher frequency means a higher pitch. The height of a sound wave determines the amplitude (or intensity) of a song. Last, the duration is the length of a song in, for example, seconds. These structural facets can be described as both relative and absolute. The relative aspects of a song indicate differences between features of elements of a song. For example, the relative amplitude of a song describes the differences between amplitudes of elements. In addition, the absolute aspects of a song describe features without comparing elements. This means, for example, an

element of a song has a certain amplitude, regardless of the differences between the amplitude of elements of this song.

Besides these structural aspects of a song, the 'voice' of a bird could be an item for memorisation. In humans, one's voice can be recognised regardless of what one vocally produces. It is still unclear of what features the voice of birds exists. Therefore, researchers focus on manipulating specific sound features of songs to determine their importance for the recognition of a song in preference tests. For example, the importance of syllable order for song recognition in zebra finches was investigated by changing this order. In these experiments, a bird was exposed to the tutor's song with changed syllable order and a novel song with changed syllable order (Mol et al., 2012). This research showed birds were still able to distinguish their tutor's song from a novel song. Thus, the ability to recognise a tutor's song is independent of syllable order. More research is needed to investigate what acoustic features of birdsong are memorised and used in recognition.

1.5 Conclusion

This chapter discussed the production and memorisation of songs in zebra finches. Only male zebra finches sing, and they learn their unique song from a tutor during a sensitive phase early in life. Also females seem to go through a sensitive phase and are able to memorise their tutor's song. Since this sex does not sing, tutor song memorisation has to serve other purposes. Both males and females are able to memorise other conspecifics' songs as well, and this takes place throughout life. The distinction between song memorisation during the sensitive phase and throughout life is important since it can provide information on the functions of song memorisation in females. The next chapters will take the timing of memorisation into account when discussing the functions of song memorisation in recognition and mate choice.

Chapter 2 Recognition

Although cues like visual appearance are used for recognition as well, this chapter focuses on the role of song in recognition. When a bird hears a song, it is compared to the songs in the memory of that bird. We hypothesise the memory of songs functions as a template. If there is a match between the heard song and the template, the bird is said to recognise the heard song. A hundred per cent match with the template is unlikely since a song sung by the same bird never sounds exactly the same. For example, a song could differ in duration or the acoustics of the environment could alter the produced sounds. How many of the heard song and the template should match to recognise it?

We distinguish two kinds of templates used in recognition: 1) 'individual templates', and 2) 'group templates'. An individual template is formed upon hearing one specific bird's song several times. When hearing this bird's song again, it has a high match with the formed template and it is recognised. This first way of recognition could be involved in individual recognition: "the signaller is recognised by unique recognition cues, and the receiver learns the cues and uses them to identify the signaller during future interactions" (Tibbetts & Dale, 2007, p. 529). These unique recognition cues could be the bird's song.

The second type of template is the 'group template', which is formed based on characteristics of several songs. When hearing a novel song that resembles this 'group template', this novel song could be recognised as belonging to this group. In this case, the match with the template is much lower compared to the match with an individual template.

Memorising a song could play a role in recognition at different levels: to recognise conspecifics, own group members, mate and kin. These levels are discussed in the following paragraphs. It probably differs per level of recognition how much the song and the template have to match to be recognised.

2.1 Conspecific recognition

Recognition in males and females may have some similar functions, although there are some differences between the sexes as well. Males probably experience some male-male competition in finding a mate, since the female is the choosing sex (Zann, 1996). For females, which are choosing their mate, it is necessary to distinguish conspecifics from members of other species. The German researcher Klaus Immelmann (1975) suggested that an own species preference is

developed by means of sexual imprinting. This has to happen early in the life of zebra finches, before pair-bonds are formed.

There are several characteristics that could be used for imprinting, for example social behaviour, visual characteristics and vocalisations. In this paragraph it is discussed whether memorising a conspecifics' song plays a role in sexual imprinting in females. In recognising conspecifics a 'conspecific template' could be formed to compare novel songs to.

Immelmann investigated sexual imprinting in zebra finches by conducting cross-fostering experiments (1969, 1972a, as cited in Zann, 1996). In these experiments he placed zebra finch eggs in the nests of bengalese finches. When these zebra finches had reached maturity Immelmann conducted mate choice experiments using live birds. It was shown that the cross-fostered zebra finch males have a preference for female bengalese finches over females of their own species. For females the results of these experiments were more difficult to determine.

From these experiments, Immelmann concluded young male zebra finches imprint on the individuals that feed them. For females, it still needs to be clarified whether sexual imprinting plays a role in choosing a mate from the same species. Besides, from these experiments it cannot be concluded what characteristics are used for sexual imprinting in zebra finches. The question remained whether memorisation of conspecifics' songs plays a role in conspecific recognition in male and female zebra finches.

More recent research has shown that the ability to recognise own species' songs in females is innate and not learned by memorising songs (Lauay et al., 2004). Research has pointed out that female zebra finches that grew up in isolation prefer conspecific to heterospecific songs (Lauay et al., 2004). Thus, hearing and memorising songs of conspecifics early in life is not necessary in preferring conspecifics to heterospecifics in females. This means there must be an innate component in the recognition of songs of conspecifics in females.

2.2 Recognising group members

It could be beneficial for a zebra finch to be able to distinguish own group members from other individuals. We should take into account that the zebra finch is a non-territorial species that lives in large flocks with high immigration rates (Zann, 1996). Because of these high immigration rates a zebra finch often encounters new individuals and could modify its behavioural responses when encountering these individuals more often (Stripling, 2003). A novel song could be a predictor for new competition for both paired and unpaired males (Dunn & Zann, 1997 as cited in Stripling et al., 2003). This paragraph discusses the question whether female zebra finches

can recognise group members and whether they do that by means of song.

Firstly, female zebra finches could recognise group members using an 'individual template', this means that she memorises each individual's song. In this way, a bird can recognise an individual from whom it heard its song before. Stripling et al. (2003) proved that males are able to memorise a conspecific's song within three hours, without behavioural reinforcement. However, this research does not show whether these males were able to memorise the song for more than one day. Females are able to add new songs to their memory throughout their life as well (Miller, 1979). It has not been shown whether they are able to do that in a comparably short time as males. Nevertheless, we can conclude song memorisation takes place throughout a zebra finch's life, also after the sensitive phase.

Secondly, group member recognition could occur by means of comparing songs to a 'group template'. A bird could form this template by memorising characteristics of the songs of its group members. Novel songs could be matched to this 'group template' in order to determine whether this novel bird belongs to the group or not. Could there be a group song that is specific for a certain group of zebra finches? This question is discussed by looking at a different kind of 'groups': subspecies, regions and flocks.

An example on the level of subspecies is provided when looking at the two zebra finch subspecies *Taeniopygia guttata guttata* and *Taeniopygia guttata castanotis*. When comparing songs of these subspecies, it is found that songs of *T. g. guttata* possess longer motifs and more elements than songs of *T.g. castanotis*, and the latter is sung at a lower pitch (Clayton et al., 1990). Cross-fostering experiments showed that females are able to discriminate between songs of the two subspecies and prefer the song of the subspecies they grew up with (Clayton et al., 1990). If a bird of one subspecies grew up in the nest of the other subspecies, the bird preferred the songs of the subspecies that it was reared in. This shows that the preference for a subspecies is a learned ability.

So on the level of subspecies birds can distinguish group members by comparing songs with a 'subspecies template'. Does this apply to other group levels, for example the level of geographical regions, as well? Zann (1993) investigated wild zebra finch song variation by comparing songs from flocks of south-eastern and central Australia. He found that songs of these two regions are distinguishable, which he ascribes to "a reduced dispersal on a macrogeographic scale" (Zann, 1993, p. 725). In this way, zebra finches can potentially match a novel song to a region-specific template to determine whether an individual belongs to the same

region or not. If zebra finches are able of distinguishing between zebra finch songs from the different regions needs to be clarified by further research.

What is known about zebra finch songs and recognition on flock level? Zebra finches live in large flocks (also called colonies). The size of these flocks depends on the abundance and distribution of food and breeding activity (Zann, 1996). Zann (1993) analysed the song variation between colonies that lived in the same region, but did not find significant differences. “Despite resemblance in song structure between sons and their fathers, dispersal to and from colonies prevents the formation of colony-specific versions of the song” (Zann, 1993, p. 716). Concluding, the immigration rates for both males and females in flocks seem too high for the formation of a flock-specific song. This means flock member recognition could only be done by individual recognition, by forming a memory of an individual’s song.

2.3 Mate recognition

Within large flocks zebra finches form long-term monogamous pair-bonds, although extra-pair copulations also occur (Zann, 1996). These bonds are maintained until one of the partners dies. In some way two partners need to recognise each other. Since a male’s song is unique, it provides a good indicator for an individual. Therefore, a likely hypothesis is that the female recognises her mate by his song. We hypothesise that a female forms a template of her mate’s song to compare heard songs to. Memorisation of the partner’s song should take place throughout life, since pair-bonds can be formed also after the sensitive phase.

Playback experiments showed females prefer their mate’s song to a neighbour’s song (Miller, 1979). In this experiment, females were exposed to both their mate’s and their neighbour’s songs prior to the test. Furthermore, in preference tests in which the mate’s song and a dissimilar or a similar song were played, females were able to recognise their mate’s song (Clayton, 1988). Concluding, females are able to recognise their partner when hearing his song. Probably other cues, like calls and visual appearance, play a role in mate recognition as well. Since females do not sing, males have to use other cues than songs to recognise their partner.

2.4 Kin recognition

It could benefit an organism to be able to perceive kinship. For example, it could play a role in mate choice or kin selection. In chapter 3, the influence of the memorisation of songs on female mate choice is discussed. This paragraph explores to what extent memorisation of songs in zebra finches is used to recognise kin.

Both 'individual templates' and 'group templates' could be used to recognise kin. A bird could memorise the songs of a specific family member and recognise this song when it is heard later. Another possibility is that a 'family template' is formed containing family-specific song characteristics. In this way, a family member whom a bird has never encountered before could be recognised.

Could a 'family template' exist? Birds that grow up in the same clutches are, as long as they are in the nest, exposed to the same calls and songs. Their tutor, from whom the males learn their own song, is probably their father (Immelmann, 1969, as cited in Zann, 1996). Therefore, we expect songs of two brothers to be quite similar. Since birds copy elements from their tutor song, we expect resemblances between the tutor's song and the songs of the male offspring as well. Concluding, song could provide information about relatedness and could serve a function in kin recognition.

As described in chapter 1, both females and males are capable of tutor song recognition (Clayton, 1988; Holveck & Riebel, 2014). Since a bird's tutor is most likely its father, this means a female recognises her father's song. In addition, the use of calls in parent-offspring and offspring-parents recognition has been studied by the use of various playback experiments. It is shown that offspring that already fledged are able to recognise their parents by their distance calls (Jacot et al., 2010; Reers et al., 2013), though parents are not able to recognise their fledged offspring by their distance calls (Reers et al., 2011). However, another research showed parents are capable of recognising their offspring by means of their begging calls the day before fledging (Levréro et al., 2009). In the mentioned experiments, the offspring was still nutritional dependent of their parents and did not reach full maturity. In this case, kin recognition probably plays a role in kin selection.

Concluding, female zebra finches are able to recognise their tutor's song. Whether other kin can be recognised by their song is not clear yet. Also, we discussed experiments in which calls were played back and in which was proven calls play a role in kin recognition. Song could be a reliable cue for recognising kin as well, but further research has to point out whether zebra finches use these vocal cues for kin recognition. Playback experiments using songs of kin and novel songs could shed light the role of song memorisation in kin recognition.

2.5 Conclusion

This chapter first described how songs could be recognised by forming memories. We

distinguished forming an ‘individual template’ and a ‘group template’. Probably it depends on the kind of recognition how much a heard song has to match these templates for the song to be recognised.

We described that the memory of songs could be used for different kinds of recognition. Both males and females can form memories of individual’s songs and use these to recognise them. Besides, templates to recognise novel songs seem to be formed or already present in female zebra finches. Recognition of songs of conspecifics seems to be partly innate and zebra finches develop a preference for their own subspecies’ songs early in life.

Furthermore, research has shown that songs significantly differ between regions, so songs from different regions should be distinguishable. Nevertheless, it needs to be clarified whether zebra finches use this song variation to discriminate individuals from different regions. A flock-specific song does not seem to exist, so songs of flock members need to be memorised one by one to recognise these songs as songs belonging to the same flock.

Research has shown that female zebra finches can recognise their mate by their song. Also, zebra finches are able to recognise their father’s song and their father’s calls, but it is not clear whether the ability to recognise other kin occurs by means of song memorisation. See table 1 for an overview of chapter 2.

Table 1 An overview chapter 2: different types of recognition and whether they occur by means of song memorisation in female zebra finches.

Type of recognition	Does research support that this kind of recognition takes place by means of song memorisation in female zebra finches?
Conspecific recognition	Yes, and partly innate ^{a, b}
Subspecies recognition	Yes, ^c
Region member recognition	No, but there is evidence for the existence of a region-specific song, ^d
Flock member recognition	No, and there is no evidence for the existence of a flock-specific song, ^d However, they are able of recognising a song of an individual, ^e
Mate recognition	Yes, ^{f, g}
Kin recognition	There is evidence that father (tutor) recognition takes place by means of songs, no evidence for recognising other kin by their song ^{h, i}

Characters in the last column refer to: **a:** Immelmann, 1969, 1972a, as cited in Zann, 1996. **b:** Lauay et al., 2004, **c:** Clayton et al., 1990, **d:** Zann, 1993, **e:** Stripling et al., 2003, **f:** Miller, 1979, **g:** Clayton, 1988, **h:** Clayton, 1988, **i:** Holveck & Riebel, 2014.

Chapter 3 Mate choice

Chapter 2 showed that song memorisation in female zebra finches plays a role in recognition. Besides influencing recognition, Riebel (2003a) argued that song memorisation in females can also play a role in sexual selection; it could influence female mate choice.

Mate attraction is seen as one of the functions of birdsong (Catchpole & Slater, 1995). This means female songbirds choose their mate based upon, amongst others, the male's song. Zebra finches form long-term monogamous pair-bonds (Zann, 1996). Even though these pair-bonds are not absolutely monogamous, the quality of the chosen partner has large effects on the female's fitness. Extra-pair males are chosen based upon male's songs as well (Riebel, 2009). Especially song memorisation early in life is thought to play a role in the formation of a preference for certain songs (Holveck & Riebel, 2014).

How is female preference for songs measured? In chapter 1 we discussed three types of preference tests: live male tests, phonotaxis tests and operant tests (Holveck & Riebel, 2006). In live male tests could be observed with whom pair-bonds are formed, so these tests could be used for testing mate choice. The phonotaxis and operant playback experiments are used to investigate whether birds recognise a song. For example, if a bird approaches the speaker from which a song is played or if this bird vocalises more often during one song compared to another song, this could indicate that the bird is able to distinguish between these songs.

It is hard to test to what songs a female is more attracted to. Live males experiments cannot be used, since we are only interested songs as stimuli that elicit responses. In song playback experiments, one should be careful in assigning conclusions regarding mate choice to the behaviour a female performs. For example, a female could approach a stimulus because she wants to mate, but other explanations for this behaviour are possible as well.

This chapter discusses what role song memorisation in mate choice plays. First, the three components of female mate choice in zebra finches are described. This is followed by possible functions of song memorisation in this choice: inbreeding avoidance and finding a locally adapted mate.

3.1 Female mate choice

What does preference for a stimulus consist of, how is it established? Riebel (2000) described three components that influence female preference: an innate component, a condition-

dependent component and a learned component. These components can differ among females, this means there is variation in female preference.

3.1.1. Innate component

A part of a female's preference for a song is innate. As described in chapter 2, preference for a conspecific song over a heterospecific song is expressed even if a female is reared in isolation (Lauay et al., 2004). Furthermore, females are born with a preference for certain song features of conspecifics' songs (reviewed by Riebel, 2009). For example, a study showed female zebra finches prefer high-amplitude songs to low-amplitude songs (Ritschard et al., 2010). A high-amplitude song could be a stronger stimulus and may convey information about the characteristics or quality of a male. Amplitude or other song features could be indicators for a male's quality. However, not many general conclusions are drawn yet on what innate song preferences females possess.

3.1.2 Condition-dependent component

Besides the innate component, the condition of a female influences her preference for a male's song. Females seem to choose males with a similar condition as themselves. In two experiments with zebra finches the female's and the male's conditions were manipulated by changing their brood size (Holveck & Riebel, 2009; Holveck et al., 2011). A smaller brood size means a better condition since, among other things, the food is distributed over less beaks. These studies showed females choose males with similar brooding size backgrounds. Furthermore, Lerch et al. (2013) found in canaries (*Serinus canaria*) that females with cutted wings have a less strong preference for good quality males than normal females. Female birds of poor quality seem to be earlier 'satisfied' with forming a pair with a poor quality male than normal females.

3.1.3 Learned component

The last component of female preference is the learned component, and this is the most important one for this thesis since song memorisation is part of it. Based on the songs a female memorises, she prefers certain songs to others. In females, hearing a song during the sensitive phase is crucial to learn to make 'a judgement' about the quality of a song (Lauay et al., 2004). Females reared without the presence of a tutor, do not prefer a normal song to an abnormal song, while tutored females do (Lauay et al., 2004). As discussed in chapter 1, males that are not exposed to a tutor song in the sensitive phase (untutored males) develop an abnormal song

(Immelmann, 1969, as cited in Zann, 1996; Fehér et al., 2009). Besides learning to distinguishing between normal and abnormal songs, we have seen in chapter 2 that memorising songs early in life contributes to the development of a preference for own subspecies' songs (Clayton et al., 1990).

Clayton (1988) showed both male and female zebra finches are able to memorise their tutor's song. However, both sexes did not memorise their tutor's song when the tutor was removed at day 25 post-hatching and the birds were kept in isolation until sexual maturity. In addition, a more recent research showed that tutor song memorisation in female zebra finches from 0 days until 35 days post-hatching or from 35 days until 65 days post-hatching both result in a preference for the tutor's songs (Holveck & Riebel, 2014). Besides, this research showed these females could memorise more than one song and from different tutors until the age of 65 days post-hatching. This ability was investigated in a laboratory setting and it is not clear whether zebra finches do this in nature as well and, if this turns out to be the case, from whom they memorise their songs. Holveck and Riebel (2014) suggest further research needs to clarify this.

Concluding, female zebra finches form a memory of their tutor's song, or perhaps of multiple songs, they heard early in life. The question 'why do female zebra finches form a memory of the songs heard early in life?' is further explored in the coming paragraphs.

3.2 Inbreeding avoidance

Breeding with close relatives is known to generally induce an inbreeding depression. This emerges because of an increased number of recessive genes that are expressed in offspring. As a result, the frequency of diseases increases and, consequently, fitness decreases. Inbreeding depressions apply especially to monogamous species, such as the zebra finch. Does song memorisation play a role in inbreeding avoidance in zebra finches?

As described in 3.1.3, female (and male) zebra finches form a memory of their tutor's song (Clayton, 1988; Holveck & Riebel, 2014). When hearing their tutor's song later in life, they can recognise it. Holveck & Riebel (2014) suggested that: "as adults, males will sing and females prefer their tutor's song" (p. 125). Probably, the tutor's song is the song of a bird's father (Immelmann, 1969, as cited in Zann, 1996). This means that females learn to prefer their father's song. If this preference would result in forming a pair-bond with their father, this would have negative consequences for fitness, since parent-offspring pairing is a form of inbreeding.

Therefore, it seems illogical that females would memorise their tutor's song in order to recognise, prefer and mate with their father.

Several researchers have used the zebra finch as a model species for inbreeding avoidance, although some of the results of these experiments contradict. Mate choice experiments have been done by putting birds together and later observe what pair-bonds were formed. Preference for close relatives over strangers has been found in experiments of Slater & Clements (1981). Burley (1990) found female zebra finches prefer cousins ($r=1/8$) to unrelated males, but did not find a preference for siblings. Other studies (f.e. Schielzeth et al., 2008) did not find preference for or avoidance of siblings as well. These studies have in common that test subjects did not grow up with the related individuals that were later presented in the tests, and it is not clear whether 'preference' indicates a sexual preference (Ihle & Forstmeier, 2013). Recent literature criticises these older experiments, for an overview see Ihle and Forstmeier (2013).

A recent study of Ihle and Forstmeier (2013) on kin recognition in zebra finches shows that they are able to recognise siblings in case they grew up together. These experiments showed zebra finches rather pair with an unrelated individual than with a sibling, they avoid inbreeding.

In the discussed research of Ihle and Forstmeier (2013) the birds could choose to form a pair-bond with live birds (siblings and novel birds). Therefore, we cannot deduce from this research what cues the birds based their choice on. Nevertheless, this means females are capable of inbreeding avoidance to some extent and perhaps avoid breeding with their father as well. On the other hand, we do not know whether tutor song memorisation in female zebra finches results in preferring to mate with their father. 'Preference' for a father's song could result in social interaction with the father, rather than having an influence on mate choice.

Furthermore, zebra finches live in large flocks with high immigration rates, and the chances of forming pair-bonds with close relatives may be not that high (Zann, 1996). On the other hand, too much outbreeding can have negative effects on fitness as well, due to for example the loss of adaptations to local conditions (Kokko & Ots, 2006). Concluding, whether (tutor) song memorisation plays a role in inbreeding avoidance needs to be clarified.

3.3 Indicator for a local mate

Another possible function of song memorisation in female song preference is that a male's song could convey information on the natal area of the male. A female could benefit from having a

mate that is from the same natal area, since he could be adapted to local circumstances. By memorising songs early in life females could develop a 'local song template' to compare novel songs to. This possible explanation of the role of song memorisation in female preference is explored in this paragraph by discussing the sharing hypothesis. The sharing hypothesis mainly focuses on males, though the line of thoughts provides insights in female songbirds too.

The sharing hypothesis attempts to answer the question why birdsong is learned instead of fully innate and Nottebohm proposed the first form of this hypothesis in 1970. This hypothesis states males learn their songs from males that are near, mostly their father, in order to 'share' songs (Beecher & Brenowitz, 2005; Beecher, 2008). Shared songs are songs that have common characteristics.

There are broadly two explanations for why sharing songs with neighbouring birds could be advantageous. The first explanation is that females benefit from the local (genetic) adaptations mates could have. Nottebohm (1970) reasoned that if males learn their song before dispersal, song is an indicator of the natal area of a male. If females develop a preference for songs heard before dispersal, females prefer songs that resemble the songs from their natal area. Whether local males indeed possess beneficial local adaptations depends on the species and needs to be investigated in zebra finches.

Another explanation for why sharing song would be beneficial are advantages in competition (Rothstein and Fleisher, 1987, as cited in Beecher, 2008). If a male's song is not shared (i.e. unfamiliar) means that this male is new in a flock. Immigrants could have disadvantages compared to old neighbours, since immigrants could predict new competition and old neighbours could perform more aggressive behaviour to them (Dunn & Zann, 1997, as cited in Stripling et al., 2003). If females choose a mate that shares songs with the rest of the group, these females choose for a mate that is not new in the group. Females would benefit from having a mate that is a better competitor, since, for example, that mate could possess more resources.

In zebra finches this explanation is not very likely since zebra finches only learn one song early in life, and they have a high immigration rate. So whether songs are alike or not does not necessarily provide information about whether a bird is new. Furthermore, male-male competition in the non-territorial species the zebra finch seems to be relatively low. Therefore, the advantages in male-male competition of a 'shared song' may not exist in zebra finches.

Also, one could argue males share songs with their neighbours, simply because those songs are heard and incorporated in the bird's repertoire (Beecher, 2008). This would mean that

sharing songs arose by coincidence and does not have a function. But this does not explain why female preference exists of a learned component.

Do male zebra finches share songs? The study of Clayton et al. (1990) on zebra finches, described in chapter 2, could be seen as an example of song sharing. They showed song 'dialects' exist in the two zebra finch subspecies and that females learn to prefer the dialect of their own subspecies. Also, Zann (1993) showed that there are significant differences between songs of different regions. This means in some way zebra finches of a subspecies and within a region share songs. The dialects in subspecies or in region could be recognised by a female and be used to determine whether a male is from the same subspecies/region.

Concluding, the sharing hypothesis, that was formulated to explain why birdsong is developed by vocal learning, provides hypotheses on the functions of song memorisation in females as well. These hypotheses state that females benefit from a local mate because of 1) local (genetic) adaptations and 2) advantages in male-male competition. Evaluating these two advantages by comparing what is known about zebra finches, it appears that there is no evidence that the sharing hypothesis accounts for the role of song memorisation in mate choice in female zebra finches.

3.4 Conclusion

Female preference for songs consists, besides an innate and a condition-dependent component, for a part out of learning. The memorisation of songs that influence preference is thought to take place early in life. This chapter described the influence of song memorisation on female mate choice. We will now summarise the findings, see figure 1 for an overview.

Memorising songs leads preferring to mate with a bird from the same species and the same subspecies. Besides, song memorisation early in life contributes to the ability to distinguish a tutored male's song from an untutored male's song. The avoidance of inbreeding is a possible explanation for the role of tutor song memorisation in female mate choice as well, but since zebra finch flocks have high immigration rates inbreeding might not play a large role.

Furthermore, song memorisation could serve a function in choosing a local mate, as proposed by the sharing hypothesis. Although 'shared songs' could exist in the form of a subspecies or regional dialect, the benefits of choosing a local mate are not clear in zebra finches. The first benefit is the local adaptations that a male could possess, but research needs to clarify whether local mates indeed possess these beneficial adaptations. Second is the benefit that a male with a shared song could be a better competitor. Zebra finches only learn

one song and there is much immigration in flocks, and therefore a male's song does not accurately indicate whether this male belongs to the same group. On top of that, it is not clear whether there are benefits of males that are better competitors, since the zebra finch is a non-territorial species with little male-male competition.

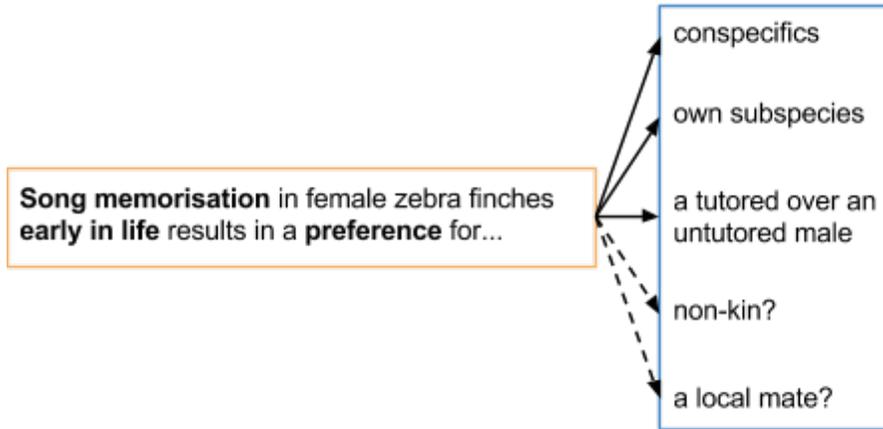


Figure 1 An overview the (possible) roles song memorisation early in life plays in the mate choice in female zebra finches, see text for explanations. A dashed arrow means more research is needed to clarify the role of song memorisation in these preferences.

Discussion and conclusion

Both male and female zebra finches are able to memorise songs early in life and throughout life. Since memorisation brings along costs, it has to have benefits to outweigh these costs. The functions of song memorisation in males probably differ from females due to the differences between the sexes. For example, males memorise their tutor's song in order to develop an own song and are the advertising sex, while females do not sing themselves and are the choosing sex. This thesis discussed the possible functions of song memorisation in female zebra finches.

We discussed recognition and mate choice as possible functions of song memorisation in females. A song can be compared to the memory of songs ('templates') and if there is a match, this song is recognised. We hypothesised novel songs can be recognised by comparing them to a 'group template'. The recognition of different accents might function alike. For example, people in the south of the Netherlands have distinguishable accents. Some memory is formed of characteristics of this kind of speech ('south of the Netherlands template') and using that, this accent can be recognised, regardless of whether you heard this person with the accent speak before. Research has shown that memorising songs plays a role in conspecific, subspecies and mate recognition. Concluding, song is one of the cues that female zebra finches use to distinguish an individual from other individuals.

Besides a function in recognition, memorised songs early in life play a role in mate choice in females. It still needs to be clarified whether song memorisation is used as a mechanism to avoid mating with close kin, although inbreeding might not play a large role in the life of zebra finches, because they have high immigration rates. Besides inbreeding avoidance, memorisation of songs could be used to find a local mate. Whether female zebra finches benefit from having a local mate with local (genetic) adaptations needs clarification. Insights into the advantages of having a local mate could also shed light on the 'good genes' hypothesis (a.o. Zahavi, 1975, as cited in Davies et al., 2012), that states organisms choose their mate for its good genes.

To further clarify the functions of song memorisation in female zebra finches more research is needed. Whether song memorisation plays a role in other kinds of recognition can be investigated using preference tests (phonotaxis or operant tests) in which different songs are played to a female. In investigating female mate choice, the difficulty remains extrapolating 'song preference' to a predictor for mate choice. If live animals would be used, one could actually observe mate choice in case pair-bonds would be formed. However, when using live

animals other mechanisms next to the memory of songs probably affect the choice for a mating partner. Possibly a combination of song playback tests (phonotaxis or operant) and live animal tests could shed light on the function of song memorisation in female mate choice.

What functions song memorisation serves depends on the species characteristics. Therefore, the functions of song memorisation zebra finches might not account for other songbird species. In zebra finches only the males sing a song that is acquired early in life. Therefore, zebra finches are called close-ended (or age-limited) learners, though open-ended learners exist as well (Beecher & Brenowitz, 2005; Bolhuis & Gahr, 2006). The latter group of songbird species experience no time constraint in expanding their repertoires. The sharing hypothesis is probably better applicable to these songbird species. When open-ended learners immigrate to a new group, they are able to adapt to this group by acquiring a shared song (i.e. a song that resembles songs of group members). Furthermore, songbird species vary in how many songs they learn and to what extent they learn their song(s) by imitation (Beecher & Brenowitz, 2005). Despite of such differences, song memorisation probably plays some role in recognition and mate choice in all songbird species.

Given that song memorisation plays a role in mate choice, it could shed light on the large variety in songbird species. If birds are isolated because of different female song preferences, this could lead to prezygotic mating barriers and eventually to speciation.

This thesis viewed birdsong as a result of sexual selection, though recent literature suggests birdsong perhaps should be viewed differently. Although in most current songbird species only the male sings, Odom et al. (2014) suggest this was different when birdsong evolved. They argue birdsong was an ancestral trait in females and they base this hypothesis on two observations. First, both male and female songbirds sing in the tropics, which contains the most songbird diversity (Kroodsma et al., 1996; Morton 1996; Slater & Mann, 2004; Fjeldså, 2014, all as cited in Odom et al., 2014). Second, female song is widespread in songbird species in Australasia (Robinson, 1949, as cited in Odom et al., 2014). In this region birdsong is thought to have originated (Barker et al., 2004, as cited in Odom et al., 2014). By ancestral state reconstruction, Odom et al. (2014) provided evidence for that birdsong is the ancestral state in female songbirds. Furthermore, research on starlings (*Sturnus vulgaris*) has shown that injecting testosterone in females resulted in these birds singing (Hausberger et al., 1995).

The findings of Odom et al. (2014) shed new light on birdsong and questions some of the premises of past research: that birdsong arose in males as the result of sexual selection.

Besides, the question is raised why in some lineages birdsong in females is lost. In context of this thesis, this could mean that the capacity to memorise tutor songs in females is a relict of the ancestral capacity to sing. If tutor song memorisation ever had a function in song learning, nowadays this function might be lost and other functions remain. However, these new insights do not explain why female songbirds are able to memorise songs throughout life.

Investigating song memorisation could provide insights on why birdsong evolved as well, since the functions of auditory learning and vocal learning are intertwined. In case of female zebra finches, auditory learning plays some role in recognition and mate choice. However, what exactly the functions of auditory learning and vocal learning are, and how these phenomena interact, needs to be clarified by future research.

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