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# Song preferences in juvenile songbirds and their relationship to vocal learning

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# Song preferences in juvenile songbirds and their relationship to vocal learning

By

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M.A. Emory University, 2015

Advisor: Donna L. Maney, PhD

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
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# Abstract

## Song preferences in juvenile songbirds and their relationship to vocal learning

By Carlos Antonio Rodríguez-Saltos

Conspecific signals are attractive to their receivers, including those receivers that will eventually become senders. Vocal learners, such as songbirds, exemplify this point; when they are juveniles, they need to attend to the vocalizations of adults to accurately imitate them. In chapter 1 of this dissertation, I reviewed evidence that conspecific song is attractive to young songbirds and elaborated on the possible mechanisms by which that song may become attractive. In chapter 2, I tested the hypothesis that the degree to which a juvenile songbird is attracted to a song predicts the quality with which the juvenile will eventually imitate that song. I tested this hypothesis by measuring relative preferences of young zebra finches (*Taeniopygia guttata*) for two conspecific songs and evaluating the degree to which these songs were imitated. The test was conducted throughout most of the sensorimotor phase of song learning, during which zebra finches practice singing. Using operant conditioning, I gave the juveniles the choice to elicit playback of the song of their father ("father song") or the song of another familiar adult ("neighbor song"). The birds elicited playback by pressing either of two keys, each one associated with a higher likelihood of playing either father song or neighbor song. I implemented a reinforcement schedule that not only allowed me to detect preference for a song, but also balanced the daily exposure of the birds to each song. Thus, I minimized any effects of exposure on learning. Immediately after isolation from caregivers, most birds preferred father song over neighbor song. Near the end of the sensorimotor phase, the vocalizations of most juveniles were more similar to father song than to neighbor song. The degree to which father song was preferred after isolation predicted the strength with which that song was imitated near the end of the sensorimotor phase. These results suggest that the attractiveness of a song early in song learning predicts the degree to which the song will be accurately imitated in adulthood. This study advances songbirds as model organisms to study the transition from being a receiver to becoming a sender.

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## TABLE OF CONTENTS

# LIST OF FIGURES

Figures Page

# LIST OF TABLES

Table Page

# CHAPTER 1

## Literature review: Conspecific song as an attractive stimulus to juvenile songbirds

This chapter presents work slightly modified from: Rodríguez-Saltos, C. A. (2017). To Become Senders, Songbirds Must be Receivers First. *Integrative and Comparative Biology*, *57*(4), 910–919.

## ABSTRACT

Courtship signals are attractive; in other words, receivers are motivated to approach courtship signals. Although the concept of a receiver is commonly associated in the literature with that of a mate seeker, young songbirds that are learning to sing by imitating conspecifics are also receivers. Juvenile songbirds are attracted to conspecific songs, which has been shown by juveniles working to hear song in operant chambers. The mechanisms explaining this attraction are poorly understood. Here, I review studies that hint at the mechanisms by which conspecific song becomes attractive. In at least some species, juveniles imitate individuals with which they have a strong social bond, such as the father. Such cases suggest that social reward plays a role in the process of song becoming attractive. In addition, experiments using birds reared in isolation from conspecific song have shown that juveniles imitate songs that have acoustic features that are typically found in conspecific song. Those studies suggest that such features are attractive to juveniles regardless of their social experience. The relative contributions of social reward and species-typical acoustic features to the attractiveness of a song can be determined using methods such as operant conditioning. For example, juvenile songbirds can be given control over the playback of songs that differ in a given attribute, such as acoustic similarity to the song of the father. The juveniles will frequently elicit playback of the songs that are attractive to them. Investigating the mechanisms that contribute to the attractiveness of conspecific song to learners will broaden our understanding of the

evolution of song as a courtship signal, because the preferences of learners may ultimately determine what will be sung to potential mates.

## INTRODUCTION

The dance of peacock-spiders, the construction of bowerbird nests, or the songs of songbirds—courtship displays are among the most spectacular and elaborate behaviors ever recorded in animals. Since one of the functions of these displays is to attract a mate-seeking receiver, researchers interested in the evolution of the displays focus on understanding the sensory biases, preferences, and motivations that drive receivers to prefer some displays over other (Andersson, 1994). One under-appreciated receiver, however, is the individual that in order to become a sender must imitate the displays of others. This receiver must choose which individuals to imitate and understanding this choice is important to form a more integrative understanding of the evolution of courtship signals. The presence of a courtship signal will depend not only on the choices that mate-seekers make, but also on what signalers choose to display to mate-seekers.

Consider juvenile songbirds, which learn to sing by imitating the songs of other birds. Song is an attractive stimulus for juvenile songbirds, which is supported by operant conditioning; when juveniles are presented with a device that triggers playback of conspecific song, they rapidly learn to operate the device (Adret, 1993; Stevenson, 1967; ten Cate, 1991). Once the task is learned, juveniles often elicit playback hundreds of times per day. Young chaffinches (*Fringilla coelebs*) and zebra finches (*Taeniopygia guttata*), for example, quickly learn to jump on perches or peck at buttons to elicit playback of conspecific song (Adret, 1993; Stevenson, 1967; ten Cate, 1991). Birds will not perform these actions for just any sound; the birds will not activate the trigger if it elicits playback of white-noise (Stevenson, 1967). It is unclear whether juvenile songbirds in an operant context prefer conspecific song over heterospecific song, nevertheless they have been shown to be more attentive to passive playback of conspecific than to that of heterospecific song (Dooling & Searcy, 1980; Nelson & Marler, 1993).

How does conspecific song become attractive to juveniles? We may not be ready yet to answer this question, but important insights have been provided by over 40 years of studies of vocal development in songbirds. I will focus on two mechanisms that explain attraction to conspecific song, both of which have received some attention in the literature. These mechanisms are: 1) imprinting on conspecific song early in life and 2) having an innate preference for conspecific song. By innate preference it is meant that the preference develops in the absence of exposure to conspecific song, regardless of how the preference may be coded in the brain or genes or whether some experience with the bird own calls is required (see Johnston, 1988).

This review is focused on species for which song development has been studied extensively, and thus is not meant to be a comprehensive assessment of factors explaining song preferences across birds. However, the factors reviewed here, imprinting on adult song and being innately attracted to conspecific song, may be common to all songbirds. At the same time, these factors do not sufficiently explain aspects of song learning that occur in some species. For example, they do not suffice to explain the psychological motivation and choices involved in copying song from territorial neighbors, which occurs, for example, in many species of sparrows (Bell, Trail, & Baptista, 1998; Wheelwright et al., 2008). Future comparative studies of song preferences during development will help shed light on those species-specific mechanisms.

Researchers studying vocal learning in species other than birds may find this review of interest. Apart from birds, vocal learning has been found only in mammals, including humans, seals, cetaceans, and bats (Janik & Slater, 1997). Our understanding of vocal learning in mammals is not as detailed as our understanding of vocal learning in birds. Research on vocal learning in mammals is not yet at the stage in which the motivations that guide learning can be understood; in order to reach that stage, model organisms need to be found that are amenable to research in the laboratory and for which the ontogeny of vocal learning has been well described. Describing the ontogeny of vocal learning in candidate mammals is currently in progress (Knörnschild, Nagy, Metz, Mayer, & Helversen, 2009; Prat, Taub, & Yovel, 2015). These studies are benefiting from methods that were initially developed to understand learning in songbirds (Doupe & Kuhl, 1999; Janik & Slater, 1997; Jarvis, 2006; Prat et al., 2015). In light of the influence that research on songbirds has had on related work on other species, it is conceivable that studies aimed at understanding the drivers of vocal learning in songbirds may pave the way for similar studies in mammals.

## THE ROLE OF EARLY SOCIAL EXPERIENCE

Parents constitute the first source of information on conspecific signals. It may thus be adaptive for birds to imprint on their parents in order to recognize conspecifics. Imprinting on parental song may be a straightforward explanation for the observation that, in at least some species of birds, this song is the one that is most often imitated (e.g. zebra finch; Zann 1996). In species of birds that do not often imitate their parents, imprinting on the parents may help to focus on conspecifics as a source for model song. In these species, song learning often occurs after birds have dispersed to sites distant to the territory of the parents (Bell, Trail, & Baptista, 1998). At those sites, conspecifics, as opposed to heterospecifics, will be the birds that are most similar to the parents.

To understand the role of filial imprinting in vocal development, few species of birds have been studied as intensively as the zebra finch. A main reason that this animal is a favorite among researchers is that it is easily bred in the lab (Zann, 1996). Because the zebra finch is a model animal used for studying the neural basis of vocal learning (Mello, 2014; Zann, 1996), understanding the role of early life experience in setting preferences for vocal learning in this species is of broad interest.

Juvenile zebra finches develop a strong attachment to their parents. This attachment persists even several weeks after the juveniles are able to feed themselves. As is the case with many other species of songbirds, zebra finches learn to sing after they become independent from their parents (Eales, 1985; H Hultsch & Todt, 2008; Zann, 1996). If the bird is allowed to remain near the father after independence, it will imitate its father’s song (in zebra finches, only the male sings) (Clayton, 1987b; Eales, 1985), although finches are capable of learning the songs of other conspecifics when the father is replaced with other adult males after independence (Eales, 1985). The tendency to copy the father's song is not explained by the father being the only model available. After independence, zebra finches are in contact with many adult conspecifics. In fact, zebra finches are gregarious; their flocks tend to be composed of hundreds of individuals (Zann, 1996). Availability of song models, therefore, is not a problem for juveniles. Yet, as shown by a study of finches in an aviary, when many potential models are available, finches tend to imitate the song of their father (Mann & Slater, 1995). The tendency to imitate the father's song can be detected even in juveniles isolated from conspecifics for five months after weaning (Clayton, 1988). The tendency to imitate father's song is not explained by a limited capacity for learning that song only. Clearly, in the zebra finch, early life experience with the father determines what juveniles will sing once they become adults.

The effect of early life experience on song imitation is further highlighted by studies of cross-fostering to heterospecifics. Zebra finches that have been cross-fostered to Bengalese finches (*Lonchura striata*) sing Bengalese finch song when they are adults (Clayton, 1987a; Eales, 1987; Immelmann, 1969; Woolley, Hauber, & Theunissen, 2010). When separated from the foster parents and given a choice between learning from an heterospecific or a conspecific adult, cross-fostered zebra finches may learn from both (Clayton, 1987a; Eales, 1987). In contrast, a finch that is reared by conspecifics imitates conspecifics only (Clayton, 1987a; Eales, 1987).

What explains the tendency to imitate father’s song? Social bonding is likely a main part of the explanation. In support of this hypothesis, zebra finches may imitate conspecifics other than the father if they develop a social bond with them (Williams, 1990). This observation merits follow-up studies in which juveniles are allowed to develop social bonds with adult males to different degrees. Then, researchers can test whether the degree of the social bond predicts the degree with which the juveniles are attracted to the songs of the adults.

## INNATE ATTRACTION TO CONSPECIFIC SONG

Birds may have an innate attraction to conspecific song. This claim can be traced back to the first studies on vocal learning in birds. William Thorpe, a pioneer in this field, studied the capacity of hand-reared chaffinches (*Fringilla coelebs*) to imitate songs of different species including their own. The chaffinches were isolated from hearing song starting when they were as young as 5 days old (Thorpe, 1958). A bird this young was assumed to be unable to memorize song. Thorpe claimed to have found support for this assumption by showing that birds isolated from conspecifics at that age sing abnormal songs when they become adults (Thorpe, 1958). When Thorpe presented the songs of several species to a group of hand-reared juvenile chaffinches, they tended to imitate chaffinch song. Thorpe claimed to have found an innate preference for conspecific song (Thorpe, 1958). Similar claims were later made for other species of songbirds (Marler & Peters, 1977, 1987).

In order for a bird to have an innate preference for conspecific song, it must have at least a crude mental representation of that song. To shed light into the structure of that representation, the songs of isolates have been analyzed. To understand why the songs of isolates are appropriate for this purpose, song learning must be understood first. Under normal rearing conditions, a songbird memorizes the song of a conspecific and uses that memory as a template on which to model its own song (Adret, 2008; Konishi, 1965). If, however, a bird has been isolated from conspecifics, its only accessible template is one that is innate. This template, in the context of song learning, is called the crude template (Catchpole & Slater, 2008; Konishi, 1965; Marler, 1970; Soha, 2017). Because the crude template is expected to resemble conspecific song to some extent, scrutinizing the song of isolates should reveal elements found in conspecific song. Indeed, the songs of isolates contain elements of conspecific song. This phenomenon is widespread among birds; these elements have been found in the isolate songs of chaffinches (Thorpe, 1958), some species of sparrows (Marler & Sherman, 1985), and zebra finches (Price, 1979). To support the claim that the songs of isolates reflect the crude template, researchers have shown that deafening birds early in life results in songs that are considerably more unstructured than the songs of isolates (Nottebohm, 1968; Price, 1979).

The crude template is hypothesized to guide juveniles in learning to sing (Catchpole & Slater, 2008; Marler, 1970; Soha, 2017). By focusing on learning sounds that match the crude template, juveniles even speed their learning of song and increase the likelihood of accurate imitation of conspecific song. Memorization of song may be the refinement of the crude template while using that song as a model (Catchpole & Slater, 2008; Marler, 1970; Soha, 2017).

As we have seen, birds are capable of learning heterospecific songs if they are cross-fostered. Nonetheless, cross-fostered birds also seem to have a bias towards imitating conspecific song. For example, juvenile zebra finches cross-fostered to Bengalese finches sometimes sing a mix of elements from zebra finch song and Bengalese finch song if the juveniles are allowed to interact with individuals of both species (Clayton, 1987a; Eales, 1987), although singing such mix is rare. Normally-reared finches never develop hybrid songs when allowed to interact with conspecifics and heterospecifics. Thus, although early social experience contributed to the cross-fostered zebra finches singing elements of Bengalese finch song, innate preferences may have contributed to some of them singing elements of conspecific song. Even the songs of cross-fostered birds that have not had experience with conspecifics often have tempo (Woolley et al., 2010) and elements reminiscent of conspecific song (Woolley & Moore, 2011), which further supports the hypothesis that the crude template of song manifests itself when a bird has not encountered conspecific songs (Marler & Sherman, 1985; Price, 1979; Thorpe, 1958).

Throughout the learning phase, deviations from an accurate copy may occur, either because of error or because of improvisation (Hultsch & Kopp, 1989; Lemon, 1975; Sturdy, Phillmore, Price, & Weisman, 1999; Tchernichovski, Mitra, Lints, & Nottebohm, 2001). Some deviations may be costly if the song is no longer attractive to mates. Having a crude template of song may help minimize this cost by constraining the variability of song. Juvenile zebra finches have been shown to partially correct deviations of abnormal song models (Fehér, Ljubičić, Suzuki, Okanoya, & Tchernichovski, 2017; Feher, Wang, Saar, Mitra, & Tchernichovski, 2009), such as songs of isolates or previous recordings of the juveniles themselves. The songs of the juveniles often resembled conspecific song more closely than did the model songs (Fehér et al., 2017; Feher et al., 2009). The juveniles may have made those corrections based on their crude template.

The idea that juveniles imitate only songs that match a crude template may seem to contradict the fact that many birds sing extensive song repertoires. In most species of birds, however, songs within a repertoire tend to share invariant, species-specific features (Payne, 1986). Some features may be common in conspecific song because they have high fitness value. Indeed, certain syllables in conspecific song are known to be highly effective at eliciting copulation solicitation from mate-seekers, as has been shown, for example, for some syllables in the songs of canaries (*Serinus canaria*) (Vallet, Beme, & Kreutzer, 1998; Vallet & Kreutzer, 1995). To a juvenile that is learning to sing, it may be adaptive to secure the learning of features that are highly effective at attracting mates. One way to secure this learning is for the features to be encoded in the crude template of conspecific song. In turn, using a crude template as a filter for learning does not necessarily imply developing small repertoires. Extensive repertoires may be developed if the bird only partially matches songs to the crude template. The crude template may be simple, yet informative enough to filter songs that contain a certain set of features (Soha & Marler, 2000).

According to the arguments presented in the above two paragraphs, innate preferences for conspecific song may be adaptive and widespread among birds. Testing for these innate preferences, however, is highly complicated, as many confounds need to be ruled out. For example, an isolated bird may learn conspecific song, in contrast to heterospecific song, not necessarily because the bird has an innate preference for conspecific song, but because that song may be easier to sing due to physical constraints on the vocal apparatus (Nelson & Marler, 1993). Physical constraints, however, have not been ruled out in any of the studies reviewed in this section thus far. In those studies, preference for conspecific song was determined by testing whether the experimental birds imitated conspecific in contrast to heterospecific song. To rule out physical constraints, however, preferences need to be evaluated in some way that does not require waiting until a juvenile starts practicing singing (Nelson & Marler, 1993).

Measuring preference in birds that have not yet started to sing has been accomplished only a few times. In two studies, hand-reared swamp sparrow (*Melospiza georgiana*) and white-crowned sparrow (*Zonotrichia leucophrys*) nestlings increased their heartbeat (Dooling & Searcy, 1980) and calling-rate (Nelson & Marler, 1993), respectively, when presented with conspecific song. These responses were selective for conspecific song over heterospecific songs. Responding in this way to a sound suggests that the sound is biologically relevant, but not necessarily attractive. A technique that is better suited to test for attractiveness is operant conditioning. To my knowledge, only in one study (Braaten & Reynolds, 1999) was operant conditioning used to test for an innate preference for conspecific song. In that study, zebra finches were cross-fostered to Bengalese finches at an early age. The preference of the juveniles was then tested. The choices given to the birds were zebra finch song and European starling (*Sturnus vulgaris*) song. The juveniles could hear either song by landing on one of two perches. A third, silent perch was also provided. During the experiment, the juveniles landed more often on the perch that played zebra finch song. The result was taken as evidence that zebra finches have an innate preference for conspecific song.

Given the importance of the study of Braaten and Reynolds to understand innate preferences in songbirds, it is worth noting that alternative interpretations exist for the results of that study. First, it is hard to conclude that the juveniles were choosing zebra finch song because of an innate preference or because it was the sound that most closely resembled the calls of the Bengalese finch foster parents. The calls and songs of zebra finches share many acoustic features (Woolley & Moore, 2011) that may not be shared with the songs of European starlings. That Bengalese finch song and zebra finch song share acoustic features is not surprising, since zebra finches are closely related to Bengalese finches (Goodwin & Woodcock, 1982). Second, with the methodology employed, it is not clear whether birds were seeking the most-played song or avoiding the least played song. Zebra finches will rarely sit quietly in a cage; more likely, they will constantly move between at least two perches. The cage in the study of Braaten and Reynolds had only three perches, the two that triggered playback of songs and a neutral perch. With this set-up, it is possible that zebra finches were landing on a perch not so much to hear song, but to change position within the cage. The birds may have landed on the finch song perch to avoid hearing starling song, if starling song was aversive. The third caveat is that, contrary to the authors’ claims, the juveniles may not have been really isolated from conspecific song early in life. This caveat is not unique to the study of

Braaten and Reynolds; it is shared with other studies that have tested for innate preferences in songbirds.

Studies looking at innate biases for learning conspecific song were done under the assumption that recently hatched birds are unable to memorize song. To support this assumption, researchers have cited evidence that hatchlings isolated completely from conspecifics develop abnormal songs as they mature (Eales, 1985; Marler, 1970; Marler & Peters, 1987; Thorpe, 1958). However, being unable to sing a song that was heard early in life does not mean that memorization did not occur at all; hatchlings may not be able memorize song well enough to later imitate it, but they may familiarize themselves with the song. Sometimes, evidence listed in support of the inability of hatchlings to memorize song included observations that the auditory system of hatchlings is not sensitive enough to discriminate sounds that adult birds are capable of discriminating (Khayutin, 1985). However, lower capacity of discrimination does not imply that the auditory system of hatchlings is incapable of discriminating any sound at all. Moreover, recent evidence suggests that songbirds are sensitive to conspecific vocalizations even before they have hatched.

Learning conspecific vocalizations while inside the egg has just recently been discovered. This learning was described in superb fairy-wrens (*Malurus cyaneus*). In this species, nestlings beg their mothers for food using calls that are shared only with her (Colombelli-Négrel et al., 2012). The call is learned when the mother vocalizes during incubation. The call is thought to be a password that nestlings must provide to show that they are not brood-parasites (Colombelli-Négrel et al., 2012). Although prenatal vocal learning in birds has been described so far only for superb fairy-wrens, being sensitive to conspecific vocalizations during embryonic development has been described in a species that is very familiar to researchers of vocal learning: the zebra finch. The growth rate of zebra finches has been shown to change according to calls delivered by the mother during incubation (Mariette & Buchanan, 2016). When environmental temperatures are higher, the mothers vocalize more frequently. The offspring of these mothers tend to be smaller in size, which may help the offspring to dissipate heat more efficiently (Mariette & Buchanan, 2016). Maternal calls during incubation are sufficient to decrease the growth rate of hatchlings. Confirmation of this effect was obtained by exposing eggs to playbacks of the calls, despite the eggs being kept at a temperature lower than that at which mothers start increasing the rate of their calls (Mariette & Buchanan, 2016). It is unknown whether this prenatal sensitivity to sounds is relevant to song learning. Nevertheless, this sensitivity should call for caution when claiming that birds less than 10 days old are too young to become imprinted on conspecific song.

The evidence for a crude template of conspecific song is ambiguous. Nevertheless, having such a template may be adaptive for a bird. Factors that may explain the adaptive value of the crude template have already been stated in this section; briefly, 1) for a juvenile songbird, having an innate template would guide and speed song learning and, 2) the crude template may constrain the songs that are imitated, so that juveniles sing songs of good quality in order to keep communication coherent among receivers and senders. Searching for innate preferences for conspecific song, therefore, remains a relevant scientific endeavor. Researchers continuing this line of research, however, should be wary of the caveats discussed in this section.

## OPERANT CONDITIONING: AN EFFECTIVE TOOL TO TEST PREFERENCES FOR SONG

Testing preferences for conspecific song before birds are able to sing is an invaluable approach that may help to eliminate potential confounds from motor constraints. Operant conditioning is an ideal type of test for this purpose, because it allows detection of a preference without requiring the bird to sing. Moreover, under this paradigm, birds must work in order to elicit a song, and thus operant conditioning is more strongly tied to motivation to hear a song than other methods for detecting preference, such as phonotaxis. Among the studies reviewed here on the nature of learning conspecific song, only in one was operant conditioning used (Braaten & Reynolds, 1999). This technique should be more widespread. In this section, I will briefly review studies in which operant conditioning was used to understand song preferences in mate-seeker receivers. Such achievements, I hope, will inspire research in which operant conditioning is used to understand song preferences in young birds that are learning to sing.

Operant conditioning has been widely used for studying song preferences in female songbirds, within the context of mate choice. These studies have revealed attributes that tend to be preferred by females in the song of a male. For example, female starlings prefer songs delivered in long bouts (Gentner & Hulse, 2000); female swamp sparrows prefer local dialects over distant ones (Anderson, 2009); and female zebra finches prefer songs delivered at high amplitude (Ritschard, Riebel, & Brumm, 2010) and songs to which they were exposed when they were juveniles (Riebel, 2000). The latter result has also been found for adult males, but it is not known whether it is true for young birds that are learning to sing.

In swamp sparrows and zebra finches, preferences for song as measured by operant conditioning tasks predict mate choice; the songs that tend to be preferred in operant conditioning tasks are also the songs that elicit the largest number of copulation solicitation displays (Anderson, 2009) or that are sung by males that tend to be chosen as mates (Holveck & Riebel, 2007). This finding establishes operant conditioning as a relevant technique to understand mate choice. Operant conditioning may also be relevant to understand the choices of juvenile songbirds about which songs to imitate.

## CONCLUDING REMARKS AND FUTURE DIRECTIONS

Conspecific song is clearly attractive to juvenile songbirds, but the psychological mechanisms that explain this attractiveness remain unclear. Developmental studies have given us clues about these mechanisms (Figure 1.1, Table 1). Many studies suggest that imprinting with a conspecific song, namely, the father’s song, and innate preferences explain the attractiveness of conspecific song to juvenile songbirds. These explanations, however, need follow-up, preferably by using techniques that allow us to directly measure the preference of songbirds and avoiding those methods that do not rule out the effect of vocal constraints. Operant conditioning is a powerful technique to achieve this goal. Juveniles have been shown to learn quickly how to operate triggers in order to hear song, and once they learn, they trigger the playback frequently. So far, operant conditioning has been used mostly as a method for juveniles to train themselves (Adret, 1993; Derégnaucourt, Poirier, Kant, Linden, & Gahr, 2013). Often, a single song is presented for this purpose. Operant conditioning, however, easily provides the opportunity for testing preferences for songs. The use of operant conditioning has been successfully applied to study preferences in mate-seeking receivers (Riebel, 2000; Riebel & Slater, 1998; Riebel, Smallegange, Terpstra, & Bolhuis, 2002). It has enabled the confirmation that birds often chosen as mates have songs that mate-seeking receivers prefer over the songs of other birds (Holveck & Riebel, 2007). Researchers should use operant conditioning to test the preferences of young birds, test whether those preferences correlate with the songs chosen to be sung in adulthood, and, in combination with manipulations of the rearing environment, to test how early life experiences affect preferences for songs.

By studying the attractiveness of conspecific song to juveniles that are learning to sing, we increase our understanding of the dynamic phenomena that allow these courtship displays to persist in nature. Mate-seeking receivers may have the last word on which displays are passed to the next generation. But the animals that acquire their displays by imitation must choose what to put on display.

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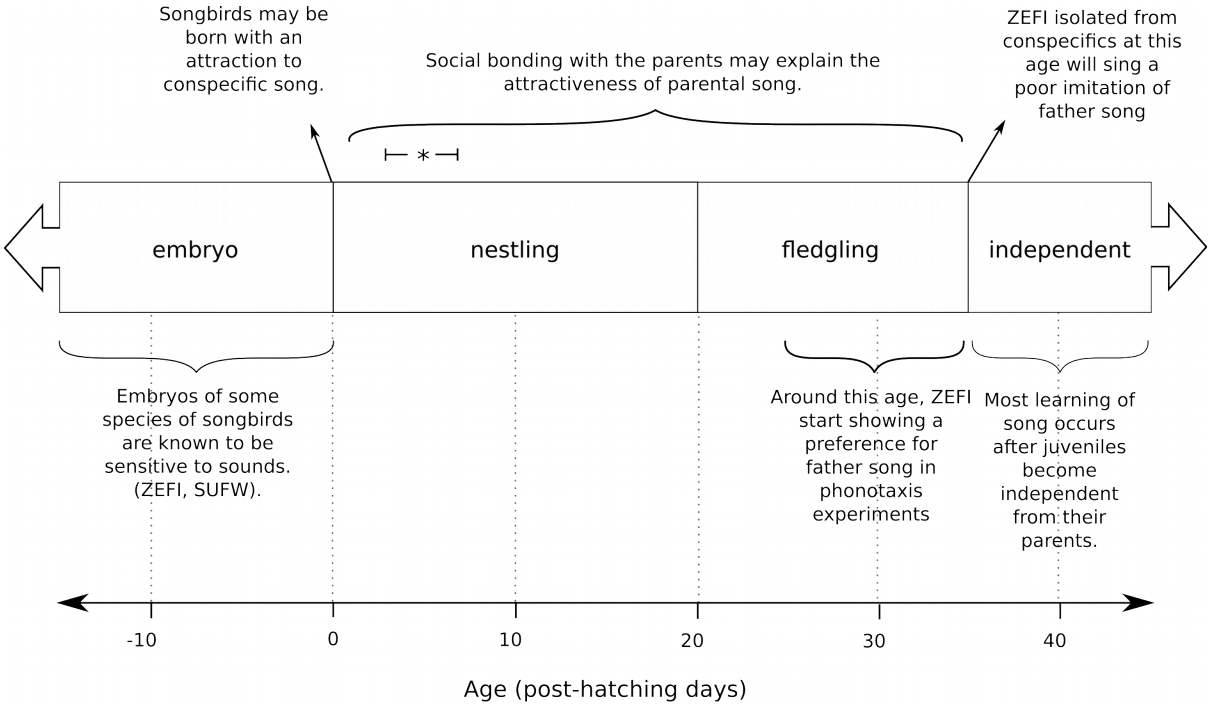

Figure 1.1: Factors that may establish the attractiveness of songs during development. The timeline is based on the development of the zebra finch, but the timing of the developmental stages closely matches that of other songbirds covered in this review (species timelines were compared according to entries in Birds of North America Online (2017) and Encyclopedia of Life (2017). A major difference across species is the time after independence at which learning of song starts (not shown). Major milestones related to song memorization are displayed below each developmental stage. ZEFI: Zebra finch, SUFW: Superb fairy-wren. * range of ages at which birds were isolated from conspecifics in studies that looked at the effects of isolation on song development and song preferences (Table 1).

Table 1: Tutor choice and preference tests covered in chapter 1. Depending on the study, the preference for conspecific song or father song was tested. All tests were conducted on birds that were already independent from their parents. Unless otherwise specified, cross-fostering consisted of transferring eggs to the foster nest. dph= days post-hatch.

| **Reference** | **Test** | **Rearing condition** | **Species** | **Mode of presentation of song** | **Choices** | **Measure** | **Results** |
|---|---|---|---|---|---|---|---|
| Braaten and Reynolds, 1999 | Preference for conspecific song | Cross-fostering to Bengalese finches, from ages 3-7 dph | Zebra finch | Operant conditioning, landing on a perch | Zebra finch song vs European starling song | Frequency of perching | Zebra finch song was preferred. |
| Clayton, 1987b | Tutor choice | Rearing by biological parents | Zebra finch | Live birds | Father vs other conspecifics | Vocal output in adulthood | Father was chosen. |
| Clayton, 1987a | Tutor choice | Cross-fostering to Bengalese finches | Zebra finch | Live birds | Zebra finch vs Bengalese finch | Vocal output in adulthood | Birds imitated elements from Bengalese and zebra finch song. |

| **Reference** | **Test** | **Rearing condition** | **Species** | **Mode of presentation of song** | **Choices** | **Measure** | **Results** |
|---|---|---|---|---|---|---|---|
| Clayton, 1988 | Preference for father song | Rearing by biological parents | Zebra finch | Passive playback | Father vs another conspecific male | Phonotaxis | Birds isolated at 35 phd, but not at 25 phd, preferred father song. The preference was detected even 5 months after of isolation. |
| Dooling and Searcy, 1980 | Preference for conspecific song | Hand-rearing from age 5 phd | Swamp sparrow, song sparrow | Passive playback | Song sparrow vs swamp sparrow | Heart rate | Swamp sparrows tended to imitate conspecific song. No clear trend for song sparrows. |
| Eales, 1987 | Tutor choice | Cross-fostering to Bengalese finches | Zebra finch | Live birds | Foster vs conspecific male | Vocal output in adulthood | Birds learned elements from both species. |

| **Reference** | **Test** | **Rearing condition** | **Species** | **Mode of presentation of song** | **Choices** | **Measure** | **Results** |
|---|---|---|---|---|---|---|---|
| Immelmann, 1969 | Tutor choice | Cross-fostering to Bengalese finches | Zebra sinch | Live birds | Bengalese finch vs zebra finch | Vocal output in adulthood | Birds learned the songs of the fosters, but if allowed to interact with conspecifics, they sometimes included elements of conspecific song. |
| Mann and Slater, 1995 | Tutor choice | Rearing by biological father in a large aviary shared with other breeding couples. | Zebra finch | Live animals | Father versus other conspecifics | Vocal output in adulthood | Father was chosen; otherwise, a bird of the same morph as that of the father was chosen. |

| Reference | Test | Rearing condition | Species | Mode of presentation of song | Choices | Measure | Results |
|---|---|---|---|---|---|---|---|
| Marler and Peters, 1977 | Tutor choice | For one group of birds, hand-rearing from ages 3-10 phd. For another group, cross-fostering to canaries starting at incubation. | Swamp sparrow | Passive playback | Song sparrow vs swamp sparrow | Vocal output in adulthood | Birds imitated only songs containing swamp sparrows syllables. |
| Marler and Peters, 1987 | Tutor choice | Hand-rearing from ages 2-10 phd | Song sparrow | Passive playback | Song sparrow vs swamp sparrow | Vocal output in adulthood | Conspecific song was imitated. |
| Nelson and Marler, 1993 | Preference for conspecific song | Hand-rearing from ages 2-7 phd | White-crowned sparrow | Passive playback | White crowned sparrow vs 5 sympatric species of birds | Call rate in response to playback | Conspecific song was preferred. |

| Reference | Test | Rearing condition | Species | Mode of presentation of song | Choices | Measure | Results |
|---|---|---|---|---|---|---|---|
| Thorpe, 1958 | Tutor choice | Hand-rearing from age 5 phd | Chaffinch | Passive playback | Chaffinch vs the multiple other species | Vocal output in adulthood | Chaffinch song was imitated. Some birds learnt pipit song, which is acoustically similar to chaffinch song. |
| Williams, 1990 | Tutor choice | Rearing by biological father in an aviary shared with other breeding couples | Zebra finch | Live birds | Father vs other conspecifics | Vocal output in adulthood | Most juveniles copied the song of adults that cared for the juveniles but were not their parents. |

# CHAPTER 2

## Relationships between the attractiveness of tutor song and vocal learning in zebra finches

This chapter has been prepared for submission to an academic journal.

## ABSTRACT

Conspecific signals are attractive to their receivers, including those receivers that will eventually become senders.  Vocal learners, such as songbirds, exemplify this point; when they are juveniles, they need to attend to the vocalizations of adults to accurately imitate them. We hypothesized that the degree to which a juvenile songbird is attracted to a song predicts the quality with which that juvenile will eventually imitate that song. We tested this hypothesis by measuring song preferences in young zebra finches (*Taeniopygia guttata*) and analyzing their adult songs.  The test was conducted throughout most of the sensorimotor phase, during which zebra finches practice singing. Using operant conditioning, We gave the juveniles the choice to elicit playback of the song of their father ("father song") or the song of another familiar adult ("neighbor song"). To elicit playback, the birds had access to two keys, and each one was associated with a higher likelihood of playing father song or neighbor song. We implemented a reinforcement schedule that not only allowed me to detect preference for a song, but also balanced the daily exposure of the birds to each song to minimize effects of exposure on learning. Near the end of the sensorimotor phase, when song was almost crystallized, we recorded hundreds of vocalizations from the juveniles and compared them to father and neighbor song. Most juveniles imitated father song to a greater extent than neighbor song. The degree to which father song was imitated was correlated with the strength of the preference for father song early in the sensorimotor phase. These results suggest that the attractiveness of a song determines the degree to which a finch will imitate it accurately.

This study advances songbirds as model organisms to study the transition from being a receiver to becoming a sender.

## INTRODUCTION

Conspecific signals are very attractive to receivers. Research on this attraction has focused mostly on signals such as courtship displays (Andersson, 1994) or foraging calls (Suzuki & Kutsukake, 2017). These signals share in common that attending to them may provide immediate benefits to the receiver. However, being attracted to conspecifics signals is also important when the benefits are not immediate. Consider vocal learners, such as humans, seals, bats, and birds (Janik & Slater, 1997; Jarvis, 2006). Juveniles of these species must attend to signals, such as speech or song, in order to imitate them; the benefits of doing so are often seen in adulthood, once these signals can be used to share information (Suzuki & Kutsukake, 2017), attract a mate, or secure a territory (Catchpole & Slater, 2008). But learning these signals may depend on juveniles being attracted to them (Rodríguez-Saltos, 2017), at a time in their lives when those benefits of signaling are beyond their reach.

Songbirds lend themselves well to studying the processes by which attraction to song contributes to vocal learning. Birds of many species can be easily reared in the lab and learn to sing in at most a few months (Catchpole & Slater, 2008). Zebra finches (*Taeniopygia guttata*), which are among the most commonly studied songbirds in the lab (Zann, 1996), are actively engaged in learning to sing. They memorize songs during social interactions with adults and their degree of attention towards tutors during these interactions predicts the quality of song imitation (Chen, Matheson, & Sakata, 2016). The need for some form of interaction during learning is further stressed by the fact that young finches do not imitate songs that are presented via passive playback; finches that have been tutored in this way produce songs that are not different from those of finches that have not been exposed to song at all (Adret, 1993; Derégnaucourt et al., 2013). Finches can, however, imitate songs if they can control playback, such as via operant conditioning (Adret, 1993; Derégnaucourt et al., 2013). Juvenile finches are rapidly lured to pressing keys that elicit playback of song, and if given the opportunity, they will elicit playback hundreds of times per day. Thus, operant conditioning has become a standard technique to train zebra finches to imitate songs chosen by researchers. The fact that young birds are willing to work to elicit playback of song shows that this stimulus is rewarding to them, just as food or drugs are rewarding to animals that are willing to press levers to obtain them.

Not all conspecific songs may be equally rewarding; finches have preferences for some tutors over others, and the songs of the tutors contribute to this choice. Zebra finches are well-suited for studies of tutor choice because each bird in this species sings only one song for life, which greatly simplifies song analysis. At the same time, there is enough variation within a population, or even a captive colony, for a juvenile to have several options of tutor song. The first song to which juveniles are exposed is that of their male caregiver —in this species only males sing. Zebra finches start practicing song once they are nutritionally independent, at 35 days post-hatch (dph). If the male caregiver is present at this time, juveniles often choose him as tutor (Mann and Slater, 1995; Zann, 1996). If he is not present, juveniles choose adults that look like the caregiver (Mann, Slater, Eales, & Richards, 1991) or sound like him (Clayton, 1987b).

It is not known whether the song of the caregiver, if presented in the absence of the caregiver himself, is preferred by juveniles over novel conspecific songs. Operant conditioning may help to answer that question. This technique has been used to study the preferences of female zebra finches for different male songs (Holveck & Riebel, 2007; Ritschard et al., 2010). The preferred songs were behaviorally relevant, in that they came from birds that also tended to be chosen as mates by females (Holveck & Riebel, 2007). In males, operant conditioning has been used to detect preferences in adults who already finished learning to sing (Adret, 1993). These adults preferred the songs with which they were tutored over the songs of other adults. Although this result is intriguing, it is unclear whether preferences in adulthood reflect preferences during learning. We do not know of any study in which more than one song was simultaneously presented via operant conditioning to juveniles, and therefore we do not know whether they prefer father song. In addition, we do not know whether such preferences play any role in learning.

The influence of attraction to tutor song on learning may depend on when the attraction occurs in development. In zebra finches, learning to sing comprises two overlapping phases. In the first one, referred to as the sensory phase, the juvenile chooses a tutor and memorizes that tutor’s song (Eales, 1985; Zann, 1996).  This phase starts at 25 dph and ends at 65 dph (Figure 2.1). Being attracted to song in the sensory phase may help the juvenile to memorize the song. In the second phase, referred to as the sensorimotor phase, the juvenile practices singing.  This phase starts at 35 dph and ends at 90 dph (Zann, 1996). Attraction to song in this phase may help the juvenile consolidate the memory of tutor song. To begin to understand the role of attraction to song in learning, it is necessary to measure preferences at multiple time points, during both phases, throughout learning.

In this study, we aimed to test the relationship between preference for a song during learning and imitation of that song in adulthood. In order to do so, we let male zebra finches be reared by their parents until a few days after the age of nutritional independence, around 35 dph (Zann, 1996). During this time, in addition to the father they could hear the songs of male adult neighbors that were housed separately and blocked from view by opaque dividers. Under these conditions, we expected the juveniles to develop a preference for the song of their father. Around age 37 dph, each juvenile was isolated in an operant conditioning cage to test his preference for the song of the father, hereafter referred to as father song, over the song of an adult neighbor that had a singing rate close to that of the father, hereafter referred to as neighbor song. We measured this preference on a daily basis until age 90 dph. In zebra finches, the span of time between 37 and 90 dph occupies most of the sensorimotor period for song learning (Zann, 1996); therefore, our design allowed us to capture this entire period. Because the sensory phase overlaps considerably with the sensorimotor phase, we were also able to measure preferences during a significant portion of that phase as well.

We predicted that the birds would prefer father song over neighbor song. To ensure that the birds had equal opportunities to learn both songs, we designed a reinforcement schedule that allowed us both to detect a preference for a song and to balance daily exposure to both songs. Our proxy for song in adulthood was the song of the juveniles recorded between 80–90 dph, when zebra finch song is almost crystallized (Johnston, 1988; Tchernichovski et al., 2001).

## MATERIALS AND METHODS

### Ethics statement

All of our procedures involving handling and experimentation with animals were approved by the Institutional Animal Care and Use Committee at Emory University.

### Finch husbandry

Adult zebra finches were randomly paired to produce offspring for our experiment. These breeding pairs were housed in 14x15x17 inch cages in the animal facility at Emory University. The birds were provided with food and water *ad libitum*. To encourage breeding, we sprayed water inside the cage on a daily basis, provided hard-boiled eggs and carrots on a weekly basis, and nesting material consisting of timothy grass and burlap as needed. All items in the cage, such as food trays, water baths, and bottles, were arranged symmetrically to discourage offspring from developing a preference for either side of the cage. Within a single room, four breeding pairs were housed together, each pair in its own cage. Birds in any cage could hear the ones in the other cages, but could not see them due to white plastic dividers placed between the cages.

### Operant conditioning

#### *Operant chamber*

Juveniles were separated from their parents at 37 dph (IQR: 36, 37) days post-hatch (dph) (Figure 2.1). By this age, juvenile zebra finches can feed themselves and they start practicing singing (Zann, 1996). We used only male juveniles for this experiment because in this species, only males sing. Each male was isolated from other birds in a 14x15x17 inch cage placed inside a sound-attenuating booth. In this cage, food and water were provided *ad libitum*. Food, water and other items in the cage were arranged symmetrically. To provide enrichment, a mirror was centered in the rear wall of the cage.

The cage was equipped with two keys (Figure 2.2, 2.3), placed on opposite walls. Upon being pressed, each key elicited playback of either father song or neighbor song from the room where the juvenile was reared. One of the keys had a higher likelihood of eliciting playback of father song while the other key had a higher likelihood of eliciting playback of neighbor song. Whether the left or right key was associated with father or neighbor song was balanced across subjects, and this association was changed only once throughout the experiment for any given bird; this change occurred if a reversal was run (see below). The keys were connected to a computer via a National Instruments Board USB-6501 (National Instruments, Austin, TX, USA) or an Arduino UNO board (Arduino LLC, Somerville, MA, USA). The computer ran the software SingSparrow!, which we wrote, to control the responses of the keys and to log the presses on them (Appendix). Each cage had two speakers, each one paired with one key. We used speakers of two models (LS-300, AudioSource, Portland, OR, USA; Logitech Z200, Newark, NJ, USA) ; in each cage, the two speakers were of the same model.

Operant conditioning proceeded daily until the bird reached 89 dph (IQR: 87, 90) (Figure 2.1), which is the age by which song is crystallized in this species (Zann, 1996). Thus, operant conditioning proceeded throughout the entire period when the zebra finches practiced singing.

### *Neighbor song*

In order to control for any effects of exposure on vocal learning, we selected a neighbor song that the juvenile heard about the same number of times as he heard father song while in the breeding room. To identify this neighbor, we estimated the singing rates of each of the four adult males in the room during the time that the juvenile was housed there—from hatching to 35-40 dph. We obtained 20-45 recordings of sounds in the room on random days and times of the day. Each recording lasted 10 minutes. Because male zebra finches sing only one song type, which is usually distinct from the song types of other males, we were able to detect each event of singing by each male just by listening to the recordings or looking at their spectrograms. We defined an event of singing as a continuous bout of song lasting for 3 seconds or less. We chose this threshold because it corresponded to the duration of the shortest bout of singing in a sample of the recordings. The average number of events per recording was our estimate of singing rate. To estimate the uncertainty in our estimate, for each male we generated an empirical sampling distribution of singing rates by bootstrapping the number of events per recording (number of bootstraps = 10000). We took this uncertainty into account when selecting neighbor song; among the three neighbors, we selected the bird for which the sampling distribution overlapped the most with that of the father.

### *Playback stimuli*

For each juvenile, we used only one stimulus as father song and one stimulus as neighbor song. Each stimulus consisted of two consecutive song motifs from the song of the corresponding bird. A motif is the unit of song; it is series of distinct sounds, known as syllables, that are sung always in the same order by a bird (Zann, 1996). To record the motifs, we separated the male from its female partner for 20 minutes. We then reunited the couple, which prompted the male to sing to the female. The song was recorded using a TASCAM DR-7MKII recorder. Sometimes, it was not possible to record two consecutive motifs without the female superimposing calls on any of them. In those cases, we used an audio editing application (Audacity) to duplicate a motif that had no superimposed calls. The stimulus was high-pass filtered at 800 Hz to eliminate low-frequency noise produced by the recording system. When Logitech Z200 speakers were (see Operant Chamber), we applied an equalization preset to the stimuli to control for frequency-dependent distortions caused by these speakers.

### *Reinforcement schedule*

In this study, we tested whether the degree to which a song is learned was predicted by the degree to which that song was preferred over another. To detect the degree to which preference predicted learning, we needed to control the amount of exposure to each song. As described above, the juveniles’ exposure to father and neighbor song was roughly equal while the juvenile lived in the breeding room. We also controlled exposure during the operant conditioning phase of the study. To do so, we designed a reinforcement schedule that allowed us to detect a preference for father or neighbor song as well as to balance exposure to each song and limit it to 30 playbacks of each song per day. This quota of playbacks was chosen to prevent detrimental effects of overexposure on learning (Tchernichovski, Lints, Mitra, & Nottebohm, 1999). It was important that the birds balanced their daily exposure to both songs; we could not balance exposure using passive playback, because passive playback leads to poor learning (Derégnaucourt et al., 2013). Each key in the operant conditioning cage was capable of playing both songs, but each had a higher probability of playing either father or neighbor song. The probabilistic schedule allowed the birds to play both songs throughout the session while still indicating their preference for one of the songs. Once the quota of their preferred song was reached, the birds could play only the other song by pressing either key, until its quota was also reached. Once the quotas of both songs were reached, finches could not elicit any more playbacks that day. The keys were reset the following morning at lights-on.

Probabilities were automatically regulated to keep the difference in exposure between the two songs to less than an arbitrary value of 11 playbacks throughout the session (Figure 2.4). The probability that a key would play its associated song changed throughout the session to prevent the preferred song from being played many more times than the other. At the beginning of the session, if the bird pressed only the key associated with his preferred song, the probability of playing that song was 0.75; in other words, out of every four presses, three resulted in playback of the preferred song and 1 in playback of the other song (Figure 2.4). The probability was this high at the beginning to strengthen the association between the key and the song, but after 12 consecutive presses, the probability went down to 0.67, and after 12 more presses, it went down to 0.5 (Figure 2.4).

When the probability of playing preferred song was 0.5, the association between the keys and the songs was maintained because under such a scenario the other key was programmed to never play the preferred song (Figure 2.4). That key played only the song with which it was associated, until that song had been heard that day the same number of times as the preferred song. In this way, the probability of hearing the associated song for each key was always much higher than hearing the other song.

When a bird switched keys, the press after the switch always resulted in playback of the song associated with the newly pressed key, regardless of how many times that song had been played. This rule was introduced to help the bird learn the associations between the keys and the songs. Moreover, if the bird did not have a preference and constantly switched between keys, exposure was naturally balanced by playing the song associated with the key being pressed.

If the bird had a preference, then the 30 playbacks of the preferred song were exhausted first. Because of the probabilistic contingencies, by the time the preferred song was exhausted, the other song had been played at least 19 times and therefore it did not take many presses to end the session. After the end of the session, the keys became silent.

### *Reversal*

For birds with strong preferences, defined as a significantly higher number of presses for one key over the other ($p < 0.05$, permutation test), we needed to rule out side biases. We did this by applying a reversal, in other words reversing the associations between the keys and the songs. Reversals were applied only once per bird and occurred during the night, in between sessions of operant conditioning.

## Recording of vocalizations

Recordings of the vocalizations of juveniles were made between 80–90 dph (Figure 2.1). By 80 dph, song crystallization in zebra finches is almost complete and song at this age is a good proxy for adult song (Johnson, Soderstrom, & Whitney, 2002; Tchernichovski et al., 2001; Zann, 1996). We used the software Sound Analysis Pro (SAP) (Tchernichovski & Mitra, 2004) to record the vocalizations. This software was developed to automatically record zebra finch vocalizations, but it does not distinguish songs from other vocalizations such as calls. Thus, our recordings included diverse types of vocalizations. When estimating imitation scores (see Estimation of imitation scores), we used an algorithm that increased the likelihood of analyzing imitations of father or neighbor song rather than other sounds produced by the juvenile.

## Data analysis

### *Reconstructing developmental trajectories of song preference*

Logs of key presses were cleaned in four steps before they were used to estimate trajectories of song preference. First, we deleted days in which the birds had not exhausted the quota of both songs. These days occurred mostly at the beginning of the experiment, while the birds were habituating to the cage. Second, we kept presses from every day only until the quota of the preferred song for that day was exhausted, because afterwards the birds no longer had a choice between two songs. Third, we removed presses made in rapid succession, because they were not independent. We had seen in videos recorded by us that finches generally do not press keys in bouts that last longer than a second. We also had programmed the keys to play song only after 3 seconds of the last initiated playback. Therefore, by retaining only presses that resulted in playback we ensured that the presses were made at least 3 seconds apart from each other, and thus, that they were sufficiently spaced to not count as part of the same bout.

To measure preference for each bird, we calculated the proportion of presses for each key on each day. The strength of the preference for a song, or simply preference for a song, was the proportion of presses for the key associated with that song. When the proportion of presses for a particular song was greater than 0.5, that song was deemed the preferred song for that day. Preferences for father and neighbor song were mutually dependent, because together they added up to 1; therefore, we only used preference for father song in our analyses.

For each bird, a smooth trajectory was fitted to the values of preference for father song via locally estimated scatterplot smoothing (LOESS) (Cleveland, Grosse, & Shyu, 1992) in R (R Core Team, 2019) (Figure 2.5a). The degree of smoothing was controlled via the span parameter, which we left at the default value of 0.75. LOESS did not assume that the dataset was bounded, which was a problem given that our data were proportions. LOESS produced values outside the range 0–1 in 4 trajectories, for an average 4.03% of their lengths. The values went down to -0.01 or up to 1.12. To keep the values bounded in the range 0–1, we converted them to 0 or 1, respectively.

Presses made after a reversal cannot indicate preference until the birds have learned the new contingencies of the keys. We took a systematic approach to determine the minimum number of days after a reversal for which data needed to be discarded.  We inspected the effect that removing an arbitrary number of days, increasing from 0 to 12, had on the shape of the trajectories of preference. The major changes in the shape occurred when we removed up to three days of data after the reversal; removing any more days, until 12 days, did not change the shape of the trajectories. We interpreted that these results to mean that it took the birds at most three days after the reversal to learn the new key contingencies. We therefore removed data from the three days after the reversal.

When analyzing developmental data, it is important to account for differences in rates of development across individuals. These differences sometimes manifest themselves as shifts in the timing of developmental landmarks across individual developmental trajectories (Ramsay & Silverman, 1997). In those cases, differences in rates of development can be accounted for by aligning the landmarks across trajectories. We found two landmarks that were shared by most of our developmental trajectories (Fig. 5b) and also noted in the averaged trajectory (Fig. 5c): a low point in preference for father song followed by a peak. The resulting visual effect was that of a letter “s” laid on its side. When it was present, this s-shaped curve occurred only once. We used the low-point, the peak, and the inflection point between the two as landmarks for aligning the trajectories. To this set of landmarks, we added the beginning and end of each trajectory. To precisely locate the low point, inflection point and peak in each trajectory we obtained the first derivative of the trajectory. In this derivative, the three landmarks correspond, respectively, to the first upward zero-crossing, the peak between that zero-crossing and the following downward zero-crossing, and the following downward zero-crossing. The derivatives were estimated analytically in R (R Core Team, 2019) from cubic splines fitted to each trajectory of preference. Once the landmarks were located, the trajectories were warped using linear piece-wise interpolation to align the landmarks of each individual trajectory with those of the average trajectory.

### *Estimation of imitation scores*

Our recordings may have included sounds other than song, because our procedure for recording sounds did not automatically distinguish song from other sounds. Thus, we will refer to our set of recordings as juvenile sounds.

Juvenile sounds and tutor songs were broken into 9-ms segments with 8-ms overlap. These length and overlap values are commonly used to design sliding windows for spectral analysis of zebra finch vocalizations (Tchernichovski & Mitra, 2004; Tchernichovski, Nottebohm, Ho, Pesaran, & Mitra, 2000). To prepare juvenile sounds and tutor songs for segmentation, periods of silence were eliminated automatically after selecting a threshold of 5% of the maximum amplitude, for the juvenile sounds and 10%, for the tutor songs. The thresholds were different because juvenile sounds and tutor songs were recorded using different equipment, and thus background levels of noise were different.

For each segment of juvenile sound, we searched for the most similar segment in father and in neighbor song. The recordings of father and neighbor song were the same used for operant conditioning. Comparisons of similarity between zebra finch sounds are typically done with spectral derivatives (Tchernichovski et al., 2000). Recently, this approach has been contested because of uncertainties in the way to weight the derivatives (Kollmorgen, Hahnloser, & Mante, 2019; Mets & Brainard, 2018). Thus, we based our analysis of similarity on comparisons of the entire power spectrum, as recommended by Kollmorgen et al. (2019). We calculated spectra for all segments via the FFT algorithm, without zero-padding, using the package *seewave* (Sueur, Aubin, & Simonis, 2008) in R. Given that each segment of sound had a duration of 9ms and a sampling rate of 44100Hz, we used 410 samples to estimate the power spectrum. We used Hanning windows and normalized the amplitude in each spectrum to fit the range 0–1.

We compared raw power spectra between juvenile and tutor segments by estimating their mutual information (Cover & Thomas, 2006) via the package *infotheo* (Meyer, 2014) in R. Mutual information was used instead of spectral correlation, which is typically used to compare spectra, because it does not assume linearity. Mutual information was normalized to the entropy of the segment of tutor song to obtain a measure of similarity to the segment in tutor song. We refer to this measure as the normalized mutual information (NMI) score, and it ranged from 0 to 1. We determined that a segment of juvenile sound was matched to a segment of tutor song if the NMI score was the highest among all comparisons between that segment of juvenile sound and every segment in tutor song.

The implementation of mutual information that we used (Meyer, 2014) requires signals that are discrete. In our case, both the frequency and amplitude domains of the power spectra were discrete. In the frequency domain, the level of discretization depended on the number of samples used to generate the power spectra, which in turn depended on the sampling rate of our recordings and the length of the segments into which juvenile sounds and tutor songs were broken. In the amplitude domain, the level of discretization was chosen to minimize bias in our NMI scores. High levels of discretization biased NMI scores towards values of 1, whereas low levels biased the scores towards values of 0. We determined the level at which NMI scores spanned most of the range from 0 to almost 1 when comparing different zebra finch sounds against each other. Because zebra finch song is composed of diverse sounds (Tchernichovski et al., 2001), we reached a fast determination by comparing all segments within the same song against each other. To reach a robust determination, we performed the procedure on five songs, four from different tutors and one from a juvenile. At the lower level of discretization tested, values of amplitude in the power spectra were divided into 2 bins, and at the upper end, they were divided into 2^16 bins. Via binary search, we determined the number of bins that resulted in the following: 1) NMI scores not equal to 1, and 2) the maximum achievable spread of NMI scores. For each of the five songs, using 65 bins met these criteria.

After estimating NMI scores, we sorted segments in juvenile sound according to whether their match to father or neighbor song had the greater score. To do this, for each segment, we calculated the difference in the NMI scores for the matches to father and neighbor song. We use the term difference NMI (dNMI) to refer to the result. A positive dNMI indicated that the sound was better matched to father song, whereas a negative one indicated that it was better matched to neighbor song. When dNMI = 0, we discarded the segment.

After sorting the segments, we estimated an imitation score for each juvenile-tutor pair. We wanted our score to reflect both quality of copying and the proportion of tutor song that was copied, in line with other measures used to estimate song imitation (Tchernichovski et al., 2000). In addition, we wanted to base our score on segments from juvenile sound that likely came from juvenile song, rather than other vocalizations, and represented attempts at imitating tutor song. In other words, we wanted to avoid the risk of biasing our scores by using non-imitative sounds made by the juvenile, such as calls, that bore some resemblance to a segment in tutor song. For each juvenile, we gathered all sound segments that were matched to a given tutor song. Then, we grouped the segments according to the position in tutor song to which they were matched. From each group, we selected the segment with the maximum dNMI value, and therefore, the one that most likely represented an attempt at imitation. We preferred to use dNMI rather than NMI values because the former penalizes segments that were relatively similar to both tutor songs. To obtain the imitation score for the juvenile-tutor pair, we averaged dNMI values from all selected segments and multiplied the result by the proportion of tutor song that was covered by those segments.

### *Testing for a correlation between preference and imitation*

We tested whether preference for a given type of song (father or neighbor), at different points in development, predicted imitation of that type of song in adulthood. Our proxy for adult song was the song of juveniles at ages 80–90 dph (chronological age). After age 77 dph, zebra finches add few variations to song (Johnson et al., 2002; Tchernichovski et al., 2001; Zann, 1996); therefore, song between ages 80–90 dph is a good proxy for crystallized song. For each tutor song, we tested one correlation for every developmental day from 38 to 90 dph. Correlations were tested using beta regression models with the package *betareg* (Cribari-Neto & Zeileis, 2010) in R (R Core Team, 2019). When we aligned our data on preference, many of the data points fell into fractional developmental days. To obtain preference scores at integer developmental ages, we interpolated trajectories of preference via LOESS in R (R Core Team, 2019). For a large portion of one trajectory, LOESS predicted values that deviated greatly from the main trend in the trajectory. The deviation occurred before a sudden bend in the trajectory. Given that all values before the bend were zero, we replaced the predictions made by LOESS with zeros.

## RESULTS

### Reconstructing developmental trajectories of song preference

Using a key-pressing assay, we measured relative preference for father song over neighbor song in young zebra finches. The birds were transferred to operant conditioning cages at a median age of 37 dph (IQR: 36, 37) and housed there until a median age of 89 dph (IQR: 87-90). This range of ages overlaps almost entirely with the developmental phase during which zebra finches are in their sensitive period for learning to sing (ages 35-90 dph, Zann 1996). Out of 17 birds, 16 engaged with the task to the point that they started exhausting the daily quota of playbacks within 5.38 ± 3.52 days (mean ± standard deviation) of being housed in the cage. The age at which they started exhausting the quota did not correlate with the age at which they were transferred to the operant conditioning cages (Spearman's rho = 0.136; p = 0.642). Only one bird did not exhaust the quota of both songs after 13 days of being housed in the cage and was therefore excluded from the study.

For the 16 birds that reliably exhausted the quota each day, we estimated the strength of preference for father song over neighbor song daily by calculating the proportion of presses on the key associated with father song. To uncover general trends in these data, we used locally estimated scatterplot smoothing (LOESS). Then, to account for differences in rates of development, we aligned the trajectories by using five landmarks. (Figure 2.5). Only two trajectories could not be aligned because they did not contain all three landmarks, and were discarded from further analysis.

Father song was on average preferred over neighbor song before developmental age 50 dph (preference for father > 0.5; 0.5 not in 95% confidence interval) (Figure 2.6). Neighbor song was then preferred between developmental ages 50 and 67 dph (preference < 0.5; 0.5 not included in CI) and father song was preferred again between ages 70 and 80 dph (preference > 0.5; 0.5 not included in CI). These two shifts in preference for one song over another, however, were not observable in some individual developmental trajectories. Some birds switched their preference only once and some did not switch at all (Figure 2.5d). For this last group of birds, a valley and a peak was still seen in the trajectory of preference, which meant that the strength of the preference oscillated, but not the preference for one song over another.

### Imitation scores

Recordings of sounds for 11 juveniles were made between chronological ages 80–90 dph. Technical complications with the recording setup did not allow us to get recordings from more birds. For each juvenile, 4.95 ± 2.36 minutes (mean ± standard deviation) of sounds were compared to father and neighbor song. Using spectral analysis in combination with an information theoretic approach to measure similarity, we calculated imitation scores for each juvenile-tutor pair. In 9 out of 11 birds (82%), the score was greater for father than for neighbor song, whereas in the 2 other birds the converse was true (Figure 2.7). The scores for father song varied more than those for neighbor song, suggesting more information content for an analysis of correlation.

In this study, it was important to balance daily exposure to each tutor song. However, very early during the assay, while they were learning the task, the birds did not exhaust the daily quota of both songs creating an imbalance for those days. We measured the accumulated imbalance during those days by subtracting the number of playbacks that resulted in neighbor song from the number of playbacks that resulted in father song. Imbalance during those days (median: 0 playbacks; IQR: -5.25, 1.00) was not correlated with imitation of father song (Spearman’s rho = 0; p = 1) or that of neighbor song (Spearman’s rho = -0.023; p = 0.95).

### Correlation between preference and imitation

To test whether the strength of the preference for a song predicted the quality of imitation of that song, we fitted beta regression models to our data on preference and imitation. One model was fitted for every developmental age, from 38 to 90 dph, and each category of tutor song (father or neighbor). We were able to use data from 11 juveniles, because for these we had trajectories that could be mapped onto developmental age and imitation scores when the juveniles were 80–90 dph old (chronological age). We found significant

($p < 0.05$) correlations between imitation scores for father song and preference for that song at developmental ages 38–40 dph (over the three days: $R^2 = 0.35 \pm 0.03$; $p = 0.035 \pm 0.012$; mean ± standard deviation). Preference at any other age did not yield significant correlations (Figure 2.8). Preference for neighbor song, at any age, was not correlated with imitation of that song (max. $R^2 = 0.16$; min. $p = 0.219$). Analyses with unaligned trajectories of preference did not result in any correlation between preference and learning.

## DISCUSSION

We tested whether the degree to which a song is preferred by juvenile zebra finches predicts the quality with which the song is eventually imitated. Most of the juveniles at age 80–90, when zebra finch song is nearly crystallized, produced vocalizations that were more similar to father song than to neighbor song (Figure 2.8). The degree to which the vocalizations were similar to father song was correlated with the strength of the preference for father song early in development. Our results show that during a time window early in development, preference for a song predicts imitation of that song in adulthood.

We detected a correlation between imitation and preference only for developmental ages 38–40 dph (Figure 2.8). The plot in figure 2.8b suggest that the $R^2$ value for age 38 dph was already on a downward trend. Thus, it is possible that the association between imitation and preference was stronger before age 38 dph. Memorization of tutor song starts as early as 25 dph (Böhner, 1990; Roper & Zann, 2006; Zann, 1996); thus, before age 38 dph, being attracted to father song  may help zebra finches to memorize it.. Because memorization of tutor song extends beyond 35 dph (Eales, 1985), attraction to father song between 38 and 40 dph may continue to enable memorization. The sensory phase, during which memorization occurs, ends near 65 dph (Eales, 1985). On this day, the trajectory of R2 values reached a low point (Figure 2.8b). Thus, the effect of attraction to tutor song on memorization may start early in the sensory phase, have its peak before nutritional independence, and continue until the end of the sensory phase, albeit with decreasing strength.

Detecting a correlation between preference and imitation was possible by accounting for differences in developmental rates across individuals. To do so, we aligned the developmental trajectories of preferences according to landmarks that were salient to us. This technique, known as landmark registration, has been used in studies of human development (Ramsay & Silverman, 1997), for example, to detect growth spurts that could not be detected using traditional cross-sectional analyses (Ramsay & Silverman, 1997). To our knowledge, our work presents the first use of landmark registration for studying developmental trajectories in songbirds. We hope that this technique will become more widespread in the research of vocal learning in songbirds.

As we mentioned, vocalizations in most of our birds were more similar to father song than to neighbor song. This result is in line with numerous studies showing that zebra finches tend to imitate the song of their caregivers (Clayton, 1987b; Mann & Slater, 1995; Mann et al., 1991; Zann, 1996). Balancing exposure did not prevent most of our zebra finches from developing vocalizations that were closer imitations of father song than of neighbor song. Previous studies in which exposure was statistically accounted for (Roper & Zann, 2006), but not balanced, suggested that exposure does not affect tutor choice. Our results support this hypothesis.

In studies in which juvenile finches were presented with multiple adult male tutors, they tended to imitate song from only one of them (Zann, 1996). Under rare circumstances, a finch may develop a song that mixes elements from more than one model song; it has happened, for example, when an adult tutor is replaced halfway through the sensorimotor phase (Eales, 1985) or when a zebra finch is cross-fostered to another species and then exposed to both zebra finches and birds of the foster species (Clayton, 1987a; Eales, 1987). For each juvenile in our study, some vocalizations resembled segments of father song and others resembled segments of neighbor song. For some birds, all vocalizations matched to one of the songs had similarity (dNMI) scores close to zero (data not shown); in these cases, the scores may be explained by chance and not learning. Similarity by chance is expected to some extent for vocalizations made by individuals from the same species. In other cases, similarity scores were high for both songs (dNMI > 0.50). Based on our observations of spectrograms of zebra finch song, similarity scores this high are likely the result of imitation. Thus, some of our birds likely imitated segments from more than one song, which is a rare occurrence in zebra finches (Zann, 1996).

If preference for a song predicts imitation of that song and if birds were capable of imitating both songs in our experiment, then, why was preference for neighbor song not correlated with imitation of that song? One possible explanation is that most birds did not choose to imitate neighbor song, but accidentally copied segments from it. Some conditions that are unique to our experiment may have allowed accidental imitation. First, we carefully balanced exposure to both songs. As we have discussed, balancing exposure did not prevent father song from being more readily copied than neighbor song by most birds. However, it may have allowed imitation of at least some segments from the less preferred song. Second, we presented song in isolation from exposure to a live tutor. In studies in which zebra finches imitated only one song, live tutors were presented to the birds. Visual cues and social interactions, resulting from exposure to live tutors, affect song learning (Chen et al., 2016; Derégnaucourt et al., 2013; Mann et al., 1991) and may bias attention towards one tutor. Given that we did not present live tutors during the sensorimotor phase, we eliminated these sources of bias. Third, given our probabilistic reinforcement schedule, both songs were often played in succession, even if the bird tried to play only one of the them. Under this circumstance, some segments from the less attractive song may have been imitated because both songs were associated in time.

Two birds imitated more segments from neighbor song than from father song. These birds were siblings, and were exposed to the same father and neighbor song. It is possible that the interactions with the parents were not adequate for the birds to choose to eventually imitate father song or that neighbor song contained segments that the juvenile zebra finches found attractive regardless of experience. Neither the father nor the neighbor were used for other birds in our experiments, therefore we do not have enough data to rule out either of these two possibilities.

What determines song preference? A few days after being isolated from their parents, most juveniles preferred father song. It is possible that preferences at that time reflected the experience of the juveniles before the isolation. Back then, the father was the only adult male with which they were allowed to interact. Thus, social interactions with the father may have made his song attractive. Learning of song is known to be affected by the quality of social interactions between adult males and juveniles (Chen et al., 2016; Williams, 1990); attraction to caregiver song may mediate, at least in part, the effect of those interactions on learning. In addition, interactions with the mother may contribute to father song becoming attractive. Juveniles are sensitive to the behavioral responses of adult females to song (Carouso-Peck & Goldstein, 2019), and it is possible that the juveniles in our experiment were sensitive to the responses of their mothers to father song. Interestingly, most birds in our study switched their preference over to neighbor song by around age 50 dph. At around this time, zebra finches also lose interest in social interactions with their parents (Adkins-Regan & Leung, 2006). Taken together, our findings and those of other studies suggest that song preferences before age 50 dph are linked to social bonds.

What mechanism explains the correlation between preference and imitation? One possibility is that attractive songs engage brain systems that mediate imitation of a song. Across several species of animals, attraction to a stimulus is regulated by neuromodulatory systems such as dopamine (Berridge, 2006). In the songbird brain, dopaminergic fibers innervate associative regions of the auditory system (Durstewitz, Kröner, & Güntürkün, 1999). Dopaminergic fibers also innervate the song system (Appeltants, Absil, Balthazart, & Ball, 2000), a series of brain nuclei that control song learning (Brenowitz, Margoliash, & Nordeen, 1997). In fact, dopamine signaling in the song system mediates correction of errors during learning of song (Gadagkar et al., 2016). Given its reach into the auditory and song systems, dopamine is a good candidate to modulate the attractiveness of song and mediate the effect of the attractiveness of song on learning.

Dopamine does not need to act alone to establish a preference for father song. Given that the attractiveness of father song may depend on social bonding, neuromodulators such as oxytocin (Goodson, 2008; Insel, 2010; Maney & Rodriguez-Saltos, 2016; Theofanopoulou, Boeckx, & Jarvis, 2017) may also be involved in establishing the preference. Oxytocin is known to promote social affiliation in a variety of species of vertebrates (Goodson, 2008; Insel, 2010). One region that expresses oxytocin receptors in the brain of songbirds is the auditory forebrain (Leung et al., 2011); these receptors are expressed in juvenile zebra finches throughout the period of vocal development  (Davis, Grogan & Maney, 2019). By acting on this region, oxytocin may increase the salience of sounds with social relevance, among which is included the song of the father.

This study was conceived under the premise that vocal learners are receivers of adult signals. Preferences for signals have been commonly studied in the context of courtship, such as the preferences of females for courtship signals from males. To evaluate preferences, we used operant conditioning, a method commonly used to evaluate the preferences of sexually receptive females. We showed that preferences for songs can be measured in zebra finches that are learning to sing and that those preferences and learning are related. Traditionally, senders and receivers have been studied separately, but in the case of vocal learners, the senders must be receivers first (Rodríguez-Saltos, 2017). This study advances songbirds as animal models to understand the mechanisms mediating the transition from being a receiver to becoming a sender.

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# FIGURES

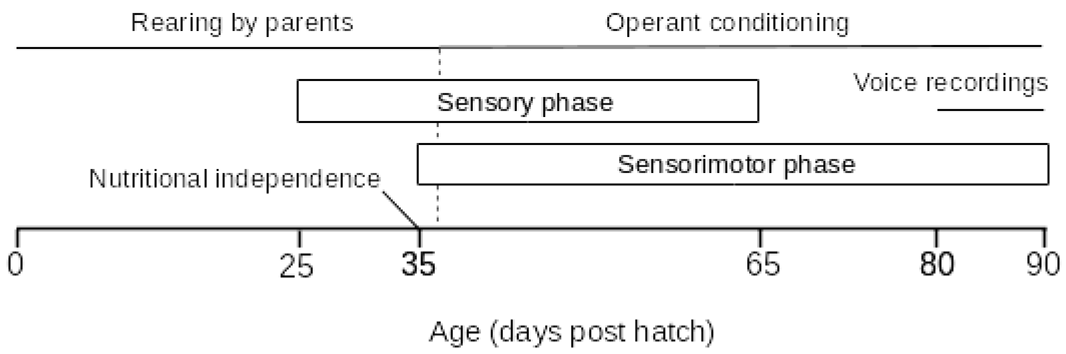

Figure 2.1: Experimental timeline. Zebra finches were reared by their parents in a room where they could also listen to, but not see or interact with, adult male zebra finches other than the father ("neighbors"). At approximately 35 dph, the juveniles were transferred to an operant chamber equipped with keys that were associated with playback of father or neighbor song. The latter was the song of an adult neighbor, the singing rate of which matched that of the father. Preference for father or neighbor song was measured daily while the bird remained in the operant chamber, until 90 dph. This age coincides with the end of the sensorimotor phase and the crystallization of song in zebra finches (Zann 1996). By 80 dph, however, learning is almost complete. We recorded vocalizations of the juveniles between 80-90 dph and compared them to father and to neighbor song.

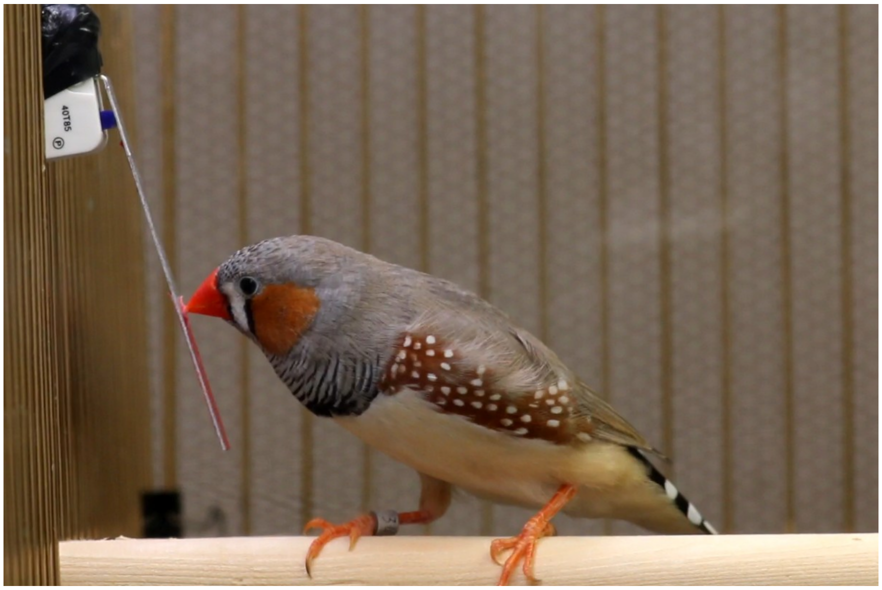

Figure 2.2: An adult zebra finch presses a key in an operant chamber to elicit playback of conspecific song. The type of key shown in the picture was used with the juveniles in our experiment. Photo by CAR-S.

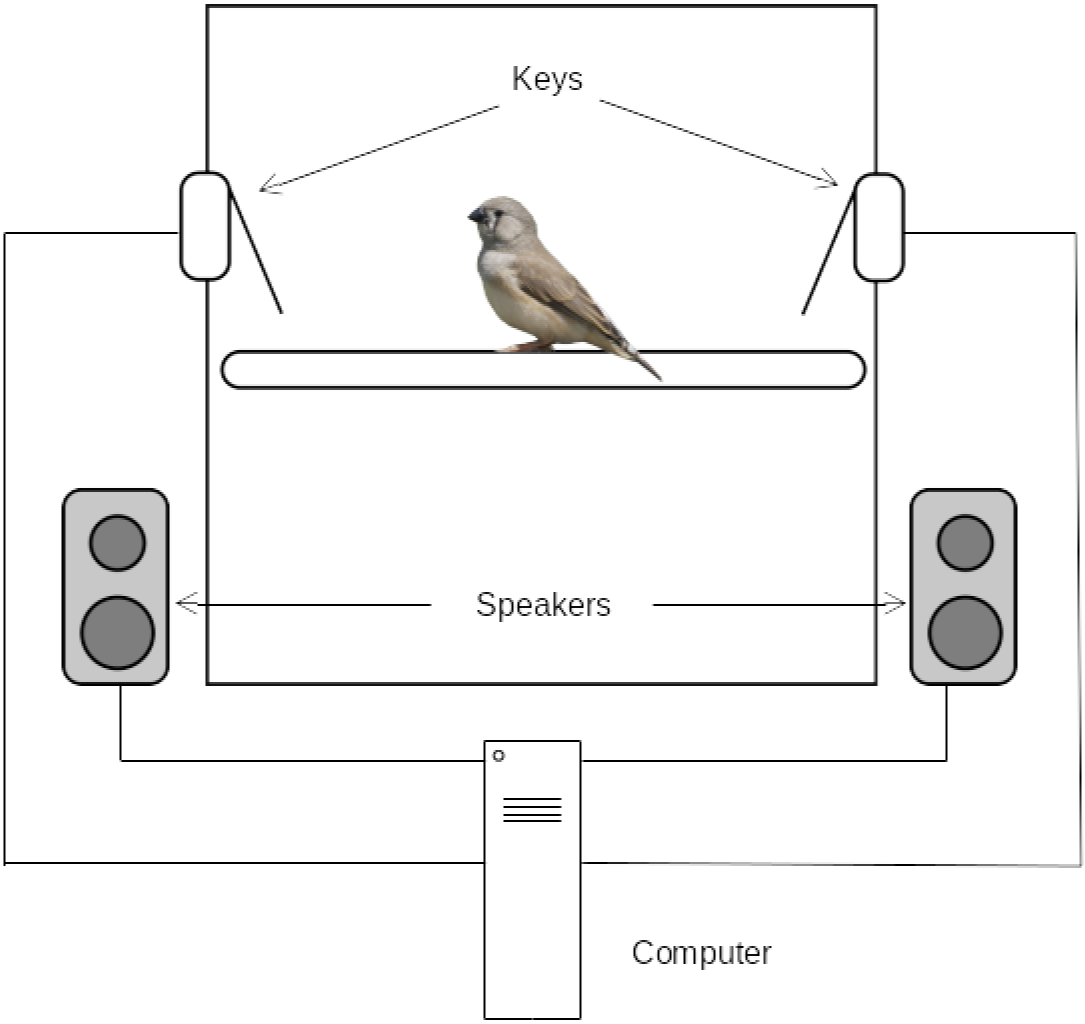

Figure 2.3: Operant set-up. The set-up consisted of a 14x15x17 inch cage, inside which two keys were placed on opposite walls. One key was associated with playback of father song and the other with playback of neighbor song. Whether the left or the right key was associated with either song was randomized across birds. The cage also contained symmetrically-arranged containers for food and water (not shown), which were provided *ad libitum*. Outside the cage, one speaker assigned to each key played the songs. Photo of finch by Lip Kee Yap, shared under the Creative Commons Attribution-Share Alike 2.0 Generic license.

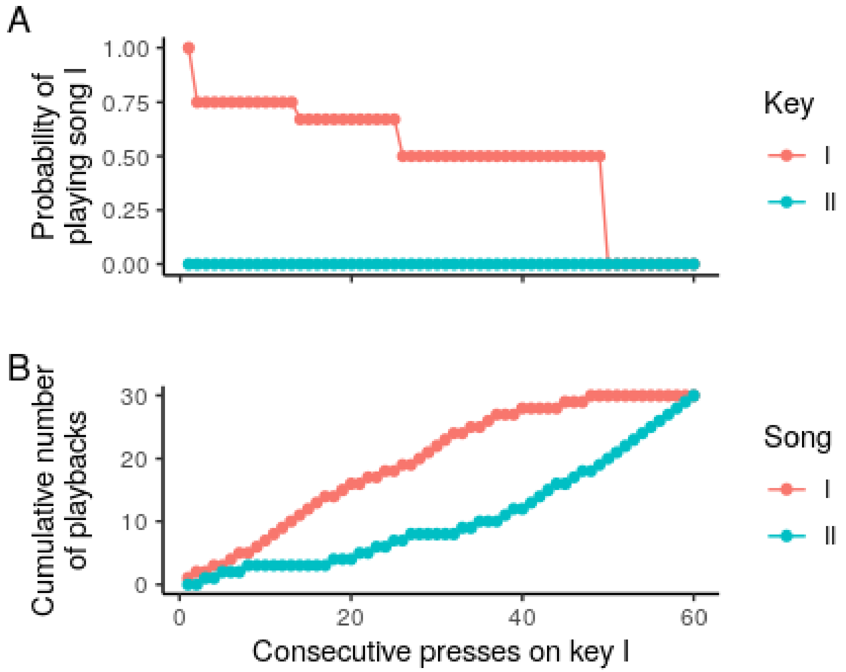

Figure 2.4: Hypothetical example to explain how our schedule allows us to detect a preference for one song over another while balancing exposure to each song. A bird is presented with keys I and II, which are associated with a higher probability of playing song I and song II, respectively. In this scenario, the bird prefers song I and presses key I only. The probability of playing song I by pressing that key is high at the beginning of the session (A), to help the bird form the association between that key and the song. As the bird keeps pressing key I, the probability decreases step-wise from 1 to 0.5, to prevent song II from lagging far behind in the playback count (B). This decrease however, is not enough to balance exposure, and therefore, if the bird switches keys, key II plays only song II until the playback count of song II is balanced with song I. After enough presses on key I, song I eventually reaches a quota of 30 playbacks and ceases to be played. Afterwards, only song II is played, until that song also reaches the quota. Importantly, there is always a large difference between the keys with respect to the probability of hearing the preferred song. When the key associated with preferred song is playing that song only 50% of the time, the other key plays non-preferred song 100% of the time.

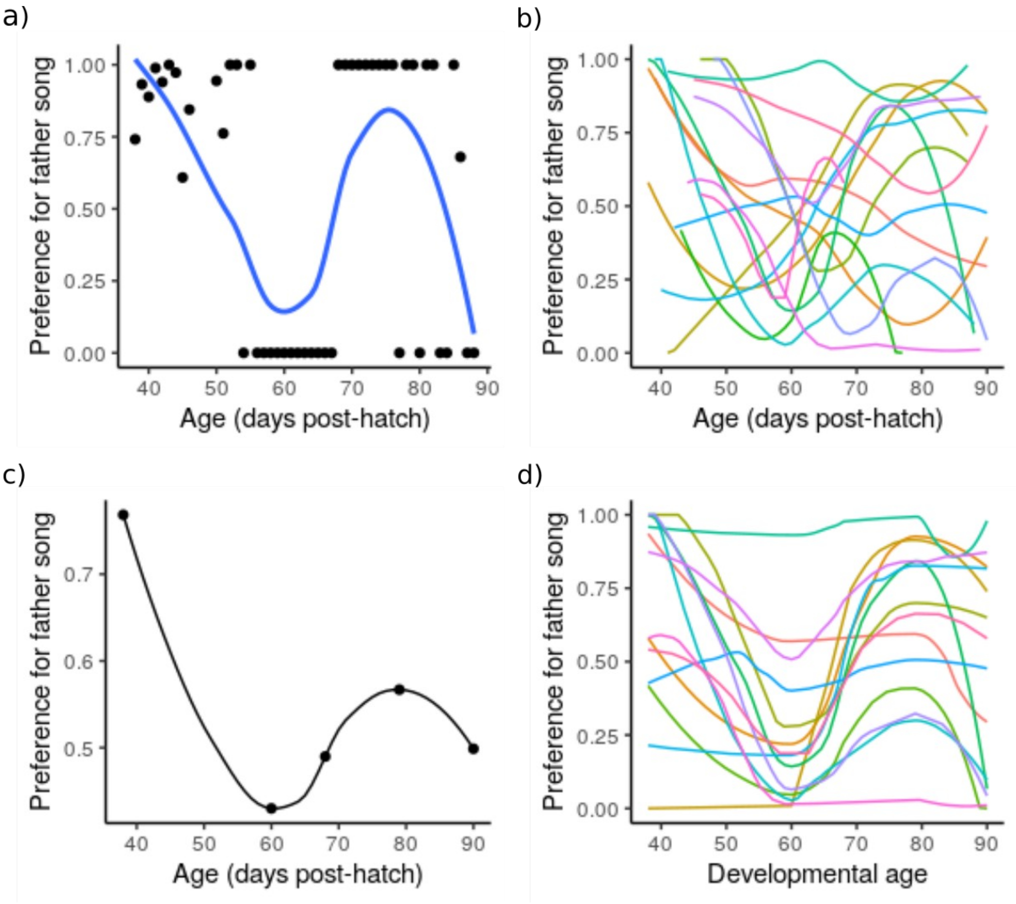

Figure 2.5: Alignment of the developmental trajectories of preference. a) For one exemplary bird, preference for father song reached a low point at 60 dph, then rebounded and peaked at around 78 dph. The dots correspond to the daily preference scores. The trajectory (blue) was obtained by applying LOESS regression to those scores. b) Trajectories of most birds also showed a valley and peak, but they occurred at different ages, probably because preferences developed at different rates across birds. We controlled for these differences by aligning landmarks in each trajectory to those in the average of all trajectories (average shown in c) (Ramsay & Silverman 1997). The landmarks were: the beginning of the trajectory; the bottom of the valley, the inflection point between the valley and the peak, the peak, and the end of the trajectory. The alignment was done using piece-wise linear interpolation. The result is shown in (d). Two trajectories that did not have both a valley and a peak were discarded.

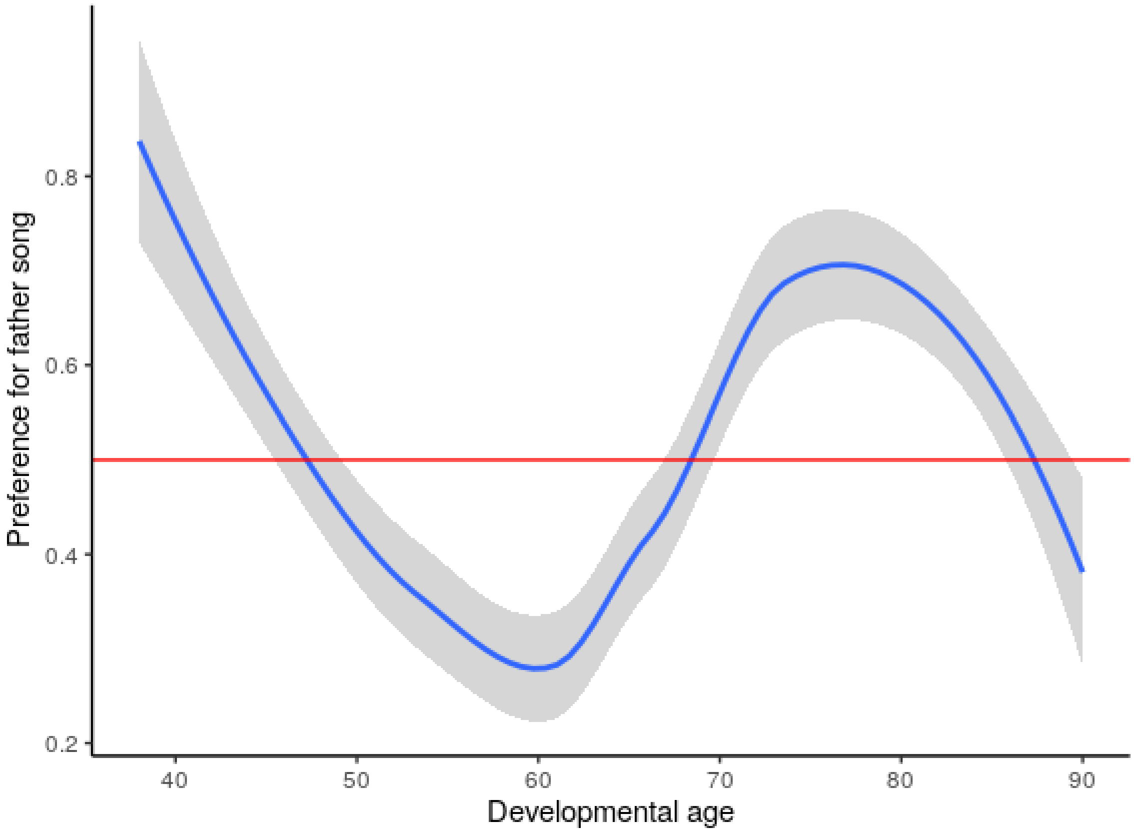

Figure 2.6: Average developmental trajectory of preference. On average, birds preferred father song over neighbor song early during development. Then, preference changed throughout development; between ages 50 and 70, the birds preferred neighbor song over father song, and after that they preferred father song again until almost age 90. The solid line is the average trajectory of preference across all birds and was obtained by applying LOESS regression to daily preference scores. The gray area corresponds to the 95% bootstrap confidence interval of the average trajectory of preference. At the red line, preference for father song is 0.5, meaning that neither father nor neighbor song is preferred over the other.

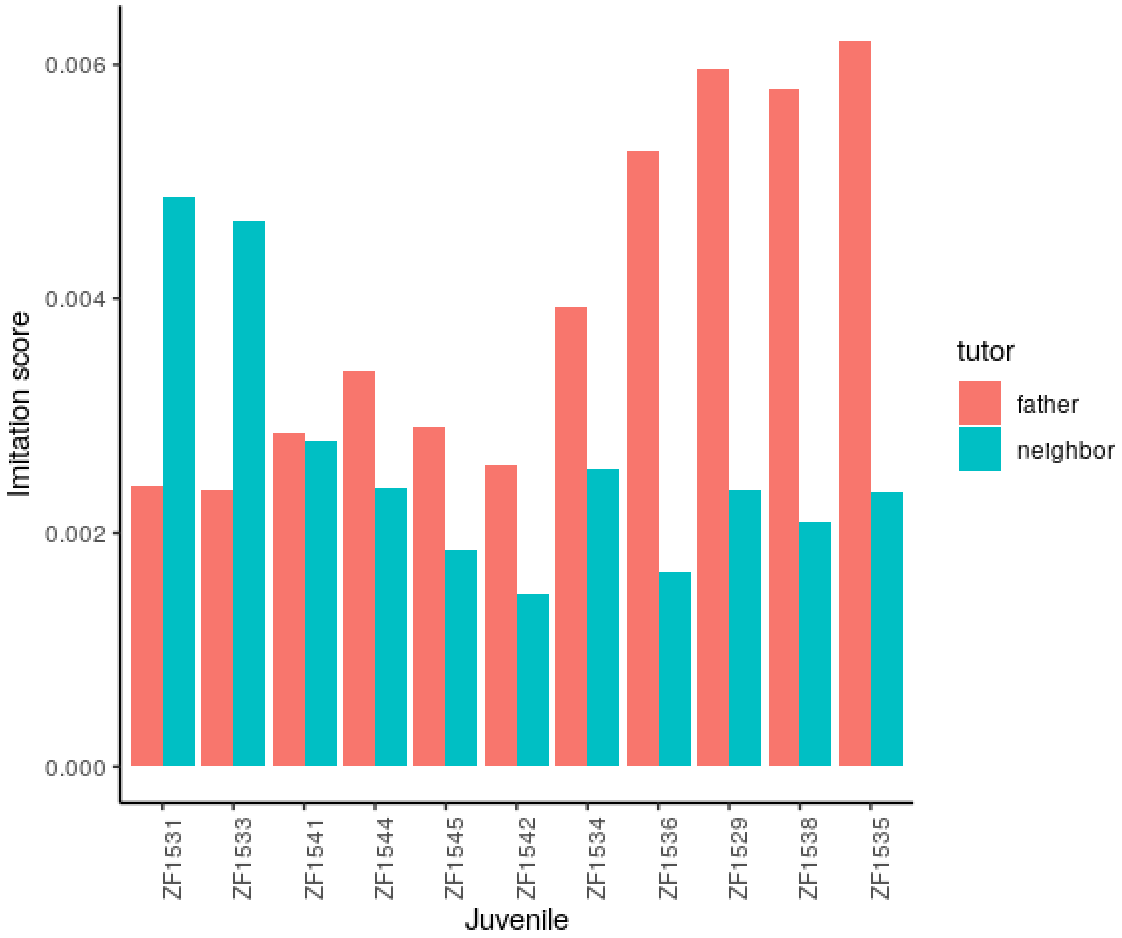

Figure 2.7: Imitation scores for father and neighbor song. For most birds (9 out 11), imitation scores for father song were higher than for neighbor song. In the plot, birds are ordered according to the size of the difference in imitation score between father and neighbor.

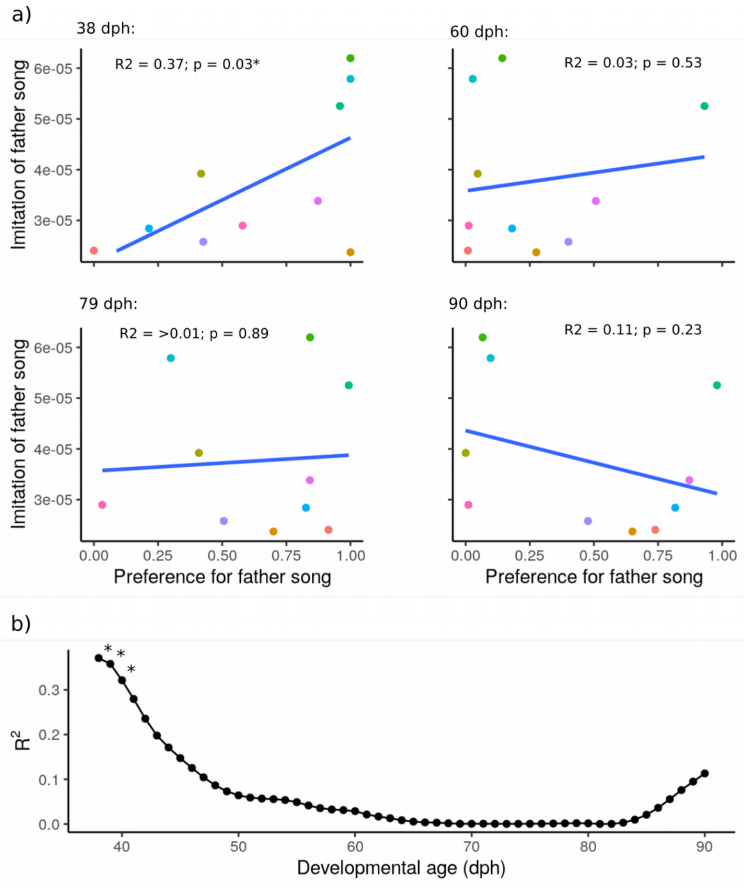

Figure 2.8: Preference for father song early in the sensitive phase for song learning predicted imitation of that song at chronological ages 80–90 dph. The relationship between imitation of father song and preference for that song at four developmental ages is plotted in (a). Ages 38 and 90 are the minimum and maximum developmental ages for which we have data on preference; ages 60 and 78 are those at which the average trajectory of preference for father song reached its lowest point and then a rebound peak, respectively. Among these ages, only at age 38 was preference for father song positively correlated with imitation scores measured at chronological ages 80–90. Each dot corresponds to one bird. Color identifies the same bird across subplots. The linear trends were inferred using a generalized linear model. R2 and p values were estimated using beta regression. R2 values of beta regression models run for every developmental age are shown in (b). Preference was correlated with imitation until 40 dph only (* $p < 0.05$)

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# Appendix

## Software developed for this dissertation

I developed the following applications for this dissertation. They allowed me to automate data collection and perform high-throughput analyses. All of the applications are open-source. Documents showing details of how these applications were used are available upon request (bio.carodrgz@gmail.com).

| **Application** | **Actions** | **URL** | **Programming languages** |
|---|---|---|---|
| SingSparrow! | Records key presses. Runs probabilistic schedules of reinforcement. | https://github.com/crodriguez-saltos/SingSparrow | MATLAB, Python, Shell |
| songPreference | Generates human-readable versions of the output from SingSparrow. | https://github.com/crodriguez-saltos/songPreference | R |
| singingRate | Estimates singing rates of birds from labeled sound files. | https://github.com/crodriguez-saltos/singingRate | R |
| somora | Estimates imitation scores. | https://github.com/crodriguez-saltos/somora | R |