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1 **Comparisons of different methods to train a young zebra finch (*Taeniopygia***
2 ***guttata*) to learn a song**

3

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17

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22

1 **Abstract**

2 Like humans, oscine songbirds exhibit vocal learning. They learn their song by imitating
3 conspecifics, mainly adults. Among them, the zebra finch (*Taeniopygia guttata*) has been
4 widely used as a model species to study the behavioural, cellular and molecular substrates
5 of vocal learning. Various methods using taped song playback have been used in the
6 laboratory to train young male finches to learn a song. Since different protocols have
7 been applied by different research groups, the efficiency of the studies cannot be directly
8 compared. The purpose of our study was to address this problem. Young finches were
9 raised by their mother alone from day post hatching (dph) 10 and singly isolated from
10 dph 35. One week later, exposure to a song model began, either using a live tutor or taped
11 playback (passive or self-elicited). At dph 100, the birds were transferred to a common
12 aviary. We observed that one-to-one live tutoring is the best method to get a fairly
13 complete imitation. Using self-elicited playback we observed high inter-individual
14 variability; while some finches learned well (including good copying of the song model),
15 others exhibited poor copying. Passive playback resulted in poor imitation of the model.
16 We also observed that finches exhibited vocal changes after dph 100 and that the range of
17 these changes was negatively related to their imitation of the song model. Taken together,
18 these results suggest that social aspects are predominant in the success outcome of song
19 learning in the zebra finch.

20

1 **1. Introduction**

2

3 Local dialects or geographical variation provided an early clue that birdsong
4 might be learned. But it was when birds were raised in controlled conditions in the
5 laboratory and their vocalizations were analyzed with the spectrograph that it was clearly
6 demonstrated (Catchpole and Slater, 2008; Zeigler and Marler, 2008). Thorpe (1954) was
7 the first to demonstrate that young chaffinches (*Fringilla coelebs*) raised in social
8 isolation failed to produce the normal song characteristic of their species. Since Thorpe's
9 pioneering works in the fifties, young oscine songbirds of several dozens of species have
10 been brought into the laboratory and reared in strictly controlled auditory environments to
11 examine how their experience affected their songs. In nearly all cases, young songbirds
12 deprived of song models develop abnormal songs, although some species-specific song
13 features may be preserved (Brainard and Doupe, 2002). It was discovered that some
14 species learn song only during a sensitive phase of early life whereas others have the
15 possibility to transform their songs during their whole life (Brainard and Doupe, 2002;
16 Catchpole and Slater, 2008). Songbird species differ also in their readiness to copy songs
17 from tape-recordings and from live tutors (for a review, see Baptista and Gaunt, 1997).
18 Some bird species learn conspecific songs from a loudspeaker easily (canaries *Serinus*
19 *canaria*: Mundinger, 1995; chaffinches *Fringilla coelebs*: Thorpe, 1958; white-crowned
20 sparrows *Zonotrichia leucophrys*: Marler, 1970; nightingales *Luscinia megarhynchos*:
21 Todt et al., 1979; marsh wrens *Cistothorus palustris*: Brenowitz et al., 1995; swamp
22 sparrows *Melospiza Georgiana*: Marler and Peters, 1988; song sparrows *Melospiza*
23 *melodia*: Marler and Peters, 1987; red-winged blackbirds *Agelaius phoeniceus*: Marler et

1 al., 1972). In other species (Treecreepers *Certhia* spp.: Thielke, 1970; Bullfinches
2 *Pyrrhula pyrrhula*: Nicolai, 1959), tutoring by tape-recording appears to be virtually
3 ineffective. Passive playback of tape-recordings has several advantages: one can control
4 the timing of sound exposure, the quality and the amount of songs that are broadcast to
5 the young bird (Derégnaucourt, 2011). This method was useful to show that some bird
6 species do learn songs during particular time windows known as sensory phases
7 (Kroodsma and Pickert, 1980). Nevertheless, it has been shown that the timing of song
8 acquisition in birds trained by passive playback could be extended beyond the limits if
9 the young bird is subsequently exposed to live tutors (Baptista and Petrinovitch, 1984).
10 Several studies show that mere proximity, that is, spatial association, is necessary for
11 song sharing (Hausberger et al., 1995) and social learning (Mann and Slater, 1995). Song
12 acquisition processes also depend on the biology of each species. For example, it is likely
13 that learning from tapes is possible in species that learn their song from their neighbours
14 under natural conditions, with whom they do not interact physically and/or visually
15 (Derégnaucourt, 2011).

16 Among songbirds, song acquisition in the zebra finch (*Taeniopygia guttata*) has
17 received a lot of interest. This tiny bird is easy to breed in captivity. Its song is short (1-
18 1.5 sec) and very stereotyped. Young male zebra finches learn their song during a
19 sensitive period of early life and it is believed that once the bird is about 90 days old, its
20 song crystallizes and will not change for the remainder of its life (Zann, 1996). Early
21 results suggested that young zebra finches would not learn from passive playback of tape
22 recordings (Immelmann, 1969; Eales, 1989). However, significant learning was observed

1 in subsequent studies despite high inter-individual variability (Bolhuis et al., 1999; Houx
2 and ten Cate, 1999a, b; Houx et al., 2000; Funabiki and Funabiki, 2009).

3 Since Immelman's pioneering study in 1969, it has been assumed that the most
4 important factor in the selection of a zebra finch song model is the close interaction
5 between a young male and the male that rears him, generally his father (Price, 1979;
6 Böhner, 1983). Indeed, this bond will even override species-specificity since zebra
7 finches will learn the song of a Bengalese finch (*Lonchura striata*) foster father, even if
8 conspecifics share their breeding accommodation. Nevertheless, in controlled conditions,
9 a young male can also learn the song of an adult male with whom he does not have
10 physical contact or social attachment such as what develops between youngsters and the
11 male that is feeding them (Eales, 1985). Still, both visual and vocal interaction between
12 young zebra finches and their tutors are important for the accuracy of song learning
13 (Eales, 1989).

14 If fairly complete song imitation in the zebra finch has never been obtained with
15 passive exposure to a song model, such results have been obtained using operant
16 conditioning with song as a reward (Adret, 1993; Tchernichovski et al., 2001;
17 Derégnaucourt et al., 2005).

18 There might be several reasons why passive playback failed so far to induce
19 significant song learning in the zebra finch. First, the song playback sessions might be not
20 done at the appropriate moment for the birds. Second, the playback quota might not be
21 sufficient to capture the bird's attention. On several occasions investigators have
22 emphasized that very good imitation is possible even when a model was presented a
23 small number of times (Petrinovitch, 1985; Hultsch and Todt, 1989; Peters et al., 1992).

1 In zebra finches, in an operant conditioning context when the bird has to peck a key to
2 trigger song playback, 40 playbacks of the song motif per day, lasting a total of 30
3 seconds, result in significant learning. More exposure leads to less complete imitation
4 (Tchernichovski et al., 1999).

5 In our study we decided to test these hypotheses. Accordingly, we decided to play
6 back song at moments that are predictable for the birds, namely, just after the lights
7 switch on in the morning and just before the lights switch off in the evening. Also, we
8 restricted the quota of daily song exposure. We will compare these results with those of
9 birds trained with a procedure of operant conditioning, and also those of live-tutored
10 birds.

11 Hormonal status is closely correlated to the end of the sensitive period for song
12 learning and the crystallization of a stereotyped song in both open-ended (Marler et al.,
13 1987) and age-limited song learners like the zebra finch (Pröve, 1983). At dph 101, we
14 measured testosterone levels and calculated correlations with song imitation, song
15 stereotypy and singing activity. Early castration (Arnold, 1975a; Bottjer and Hewer,
16 1992) and exogenous testosterone (Korsia and Bottjer, 1991) both disrupt song learning
17 in the zebra finch. In the same species, several studies suggest that high testosterone
18 concentrations reduce the potential for vocal plasticity (Korsia and Bottjer, 1991;
19 Williams et al., 2003). We therefore expected that birds with low levels of testosterone
20 would be less likely to have a stereotyped song. Also, castration of male finches
21 decreases song rates, but normal singing activity is re-established after the castrated birds
22 have been injected with testosterone (Arnold, 1975b; Pröve, 1974). In our study, we

1 checked whether there could be a link between testosterone levels and singing activity at
2 dph 100.

3 After dph 101, the birds were placed in a common aviary with conspecifics.
4 Several weeks later, their song was recorded again to quantify eventual vocal changes.

5

6 **2. Materials and methods**

7

8 *2.1. Study subjects*

9

10 62 male zebra finches from 16 different breeding pairs of our colony were used
11 for this study. Breeding pairs were maintained in separate cages (100 x 50 x 50cm) and
12 nest control was made daily. The chicks were raised by both parents until dph 10 when
13 the father was removed. The mother and the chicks were moved to another room in our
14 institute, to avoid any imprinting of the young on male song. The breeding cages were
15 maintained on LD 12:12 until the chicks fledged.

16

17 *2.2. Experimental groups*

18

19 On day 35, each chick was marked and the young males were isolated in an
20 individual sound proof chamber (LD 12:12; lights on from 8am to 8pm). Food and water
21 were provided *ad libitum*. Each bird stayed in social isolation until day 100 post-
22 hatching.

23 Birds in group 1 to 8 started training from day 43 (Table 1).

1 Birds in group 1 to 4 were exposed to a passive playback of 20 song models.
2 Birds in group 5 to 7 were trained with an operant conditioning procedure, with a daily
3 exposure to 20 song models.
4 For groups 1 and 5, there were two daily playback sessions: one in the morning (starting
5 at 8am) and one in the afternoon (starting at 2pm). During each playback session birds in
6 group 1 were exposed to a passive playback of 10 songs, and only the first 10 key pecks
7 were reinforced with song playback for group 5.
8 For groups 2 and 6, there was only one playback session in the morning, starting at 'lights
9 on.' Birds in group 2 were exposed to a passive playback of 20 songs and birds in group
10 6 could peck the key only until 2pm.
11 For groups 3 (passive playback) and 7 (operant conditioning), there was only one
12 playback session in the afternoon of 20 songs, starting at 2pm.
13 Group 4 was exposed to the passive playback of 20 songs that ended one minute before
14 'lights off.'

15

16 Some birds that trained using operant conditioning were subsequently used as
17 song tutors for the live tutoring group (group 8). On day 43 of the pupil's life, one of
18 these adult males was introduced to the sound-proof chamber and stayed with the pupil
19 until dph 100.

20

21 After dph 100, birds were moved to a common aviary where they could freely
22 interact with other males from the different experimental groups.

1 Several weeks to several months after the end of training, the birds were moved to sound-
2 proof chamber again for song recordings. After a week, they were moved back to their
3 aviary.

4

5 *2.3. Song recording and analysis*

6

7 *2.3.1. Song recording*

8 Vocal activity of each individual bird was recorded using the Sound Analysis Pro
9 software (SAP 2.0; Tchernichovski et al., 2004). The program was run on a PC equipped
10 with an Edirol UA1000 sound card (16 bits, sampling frequency: 44.1 kHz), connected to
11 multidirectional Earthworks TC20 microphones (one per sound box) placed above the
12 cage. Each bird's vocalizations were monitored continuously. To limit the recording of
13 cage noises, we adjusted the settings of the software (amplitude and duration thresholds)
14 such that mainly songs would be automatically identified and recorded.

15 Song files were then scanned using Sound Explorer (sound analysis software developed
16 by René Jansen, University of Amsterdam) and only files containing song bouts were
17 used for subsequent analysis.

18

19 *2.3.2. Singing activity*

20 Sound files recorded at dph 100 were visually scanned using Sound Explorer.
21 Only files containing song bouts were kept for the analysis. Using SAP we calculated the
22 duration of each of these song bouts to get an overall score of singing activity for the
23 whole day.

1 2.3.3. *Motif analysis*

2 We quantified how well pupils had copied their song model using a similarity
3 score procedure in SAP (Tchernichovski et al., 2000). The overall similarity score is a
4 product of 3 components: % similarity, mean accuracy and sequential match. Percentage
5 of similarity is the percentage of tutor's sounds included in final sections. Mean accuracy
6 is the average local similarity score across final sections. Sequential match is calculated
7 by sorting the final sections according to their temporal order with reference to the song
8 model, and then examining their corresponding order in the song motif.

9 For the live tutoring procedure, overall similarity score was calculated from 100
10 asymmetric pairwise comparisons of the pupil's typical motif (10 exemplars per bird)
11 with the tutor motif (10 exemplars per bird). For the playback procedures, overall
12 similarity score was calculated from 10 asymmetric pairwise comparisons of the pupil's
13 typical motif to the song model broadcast. In asymmetric comparisons the most similar
14 sound elements of two motifs are compared, independent of their position within a motif.
15 The smallest unit of comparison is 9.26-ms-long sound interval (FFT windows). Each
16 interval is characterized by measures for five acoustic features: pitch, FM, amplitude
17 modulation (AM), Wiener entropy, and Pitch Goodness. SAP calculates the Euclidean
18 distance between all interval pairs from two songs, over the course of the motif, and
19 determines a p-value for each interval pair. This p-value is based on p-value estimates
20 derived from the cumulative distribution of Euclidean distances across 250,000 sound
21 interval pairs, obtained from 25 random pairs of zebra finch songs.

22 Neighboring intervals that pass the p-threshold value ($p = 0.1$ in this study) form larger
23 similarity segments (70 ms). The amount of sound from the song model that was included

1 into the similarity segments represents the similarity score; it thus reflects how much of
2 the material of the song model was found in the pupil's motif. To measure how
3 accurately pupils copied the sound elements of the song model, we used the accuracy
4 score from SAP. The accuracy score is computed locally, across short (9 ms) FFT
5 windows and indicates how well the sound matched to the sound in the song model. SAP
6 calculates an average accuracy value of the motif by averaging all accuracy values across
7 the similarity segments.

8

9 *2.3.4. Song stereotypy at dph 100*

10 We used two methods to measure song stereotypy: (1) a measure based on a
11 clustering procedure of sounds; (2): a measure of song syntax stereotypy based on
12 amplitude shapes of singing bouts.

13 Measure of clustered sounds: we used the 'clustering' procedure of the SAP software
14 (Tchernichovski et al., 2004). The original purpose of the 'clustering' procedure of SAP
15 is to detect syllable types and to automatically trace vocal changes during song
16 development. The nearest-neighbor hierarchal clustering method is implemented into an
17 extensive graphic user interface including a display of clusters in color code, assessment
18 of residuals, and an account of the number of members in each syllable type. In our
19 study, song bouts recorded on dph 100 were segmented into syllables based on a
20 threshold crossing of song power. We then ran the clustering procedure and took into
21 account the % of residuals, namely the proportion of sounds that could not be attributed
22 to any syllable type according to the clustering algorithm.

23

1 Syntax analysis: Measures of song syntax stereotypy have been designed for zebra finch
2 song (Scharff and Nottebohm, 1991). They require manual annotation of song sequences
3 of syllables following visual observation of spectrograms. This task is relatively easy for
4 adult birds with well-defined types of syllables, but is harder when song still contains
5 plastic and unclassifiable sounds, which was the case in our study. To measure syntax
6 stereotypy we selected for each bird ten song bouts and extracted 1 sec of sound starting
7 from the 1st syllable after the last introductory note. Then, following a modified version
8 of a method recently published (Kojima and Doupe, 2011), the continuous time course of
9 the amplitude envelope was compared among these 10 1-sec segments to obtain
10 correlation coefficients (CCs) using a custom-made program written in Matlab. Finally,
11 we took the mean of these 100 comparisons. We expected that the mean CCs among
12 sound segments would be higher in finches that produce stereotyped sequence of
13 syllables than in finches that produce more variable song sequences.

14

15 *2.4. Song playback*

16

17 We used three different song models (SM1, SM2 and SM3) for playback (Fig.
18 1A-C). Each song model consisted of two identical repetitions of a single song motif
19 recorded from an adult bird. The number of birds trained with each song model is
20 presented in table 1.

21 Three finches trained with SM1, 3 finches trained with SM2 and 4 finches trained with
22 SM3 were used as song tutors for the live tutor group (group 8).

23

1 2.5. *Hormone analysis*

2

3 The absence of adult males for finches trained with song playback and the
4 isolated housing conditions are both likely to have an effect on the circulating hormone
5 levels of the young developing males (Pröve, 1981; Livingston et al., 2000), which could
6 affect song learning and song stereotypy.

7 At dph 101, a blood sample (approx. 120 µl) was taken after venipuncture from
8 the wing vein and collected into heparinized capillaries. Plasma was immediately
9 separated by centrifugation and samples were then stored at -80°C. Testosterone (T)
10 concentration was determined by direct radioimmunoassay (RIA; Goymann et al., 2006).

11

12 2.6. *Statistics*

13

14 We used a one-way ANOVA to compare learning success (similarity score with
15 the song model) between the three methods: live-tutoring, operant conditioning and
16 passive playback. The Tukey HSD test was used for post-hoc analysis. We used a two-
17 way ANOVA to test the effect of time and song model on learning success for birds
18 trained with passive playback and operant conditioning. The t-test was also used to
19 compare learning success between two training schedules for the same method. We used
20 linear regression to test correlations between different measures (similarity score at
21 100dph, similarity score after a 2nd recording, singing activity, T level and other acoustic
22 measures). General linear models were used to check whether there was a significant
23 difference among training procedures regarding these correlations. We used the

1 Kolmogorov-Smirnov test to check whether the data conformed to a normal distribution.

2 We conducted all analyses in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

3

4 **3. Results**

5

6 *3.1. Recordings at dph 100*

7

8 *3.1.1. Song imitation*

9 We observed significant differences among tutoring regimes (Fig. 2A, one-way
10 ANOVA, $F(2,59) = 15.691$, $P < 0.0001$). Birds trained with a live tutor learned better than
11 birds trained with operant conditioning (Tukey HSD, $P = 0.005$) and passive playback
12 ($P < 0.001$). Birds trained with operant conditioning did also learn better than birds
13 trained with passive playback ($P = 0.004$). Close-to-perfect imitation of the song model
14 was observed in finches trained with live tutoring and operant conditioning but not in
15 finches trained with passive playback (Fig. 1).

16

17 *3.1.2. Effect of song model and time of playback groups*

18 For both groups (passive playback and operant conditioning), we did not observe
19 significant differences among song models on the overall similarity score (song model:
20 $F(2,51) = 0.877$, $P = 0.423$; interaction song model*method: $F(2,51) = 1.423$, $P = 0.251$).

21

22 For both groups there was also no effect of time of playback on song learning.

23

1 For the operant training group, birds trained with two daily sessions learned better
2 than birds trained with one single daily session starting at lights on (Fig. 2B, t-test,
3 $t(27.812) = -2.198, P = 0.036$).

4
5 For the passive playback group birds trained with song playback at the beginning of the
6 light phase (one minute after lights on) did not learn better than birds trained at the end of
7 the light phase (one minute before lights off) (Fig. 2C, t-test, $t(13) = 2.036, P = 0.063$).

8 9 *3.1.3. Singing activity*

10 Regarding singing activity, we did not find a significant correlation with the
11 overall similarity score (Fig. 3A, linear regression, $F(1,58) = 0.17, R = 0.054, P = 0.682$),
12 meaning that birds that did learn well were not the ones singing more at dph 100.

13 14 *3.1.4. Testosterone levels*

15 Regarding T level, there was no significant correlation either with overall
16 similarity score (Fig. 3B, $F(1,50) = 0.789, R = 0.125, P = 0.379$) or singing activity (Fig.
17 3C, $F(1,50) = 2.285, R = 0.209, P = 0.137$).

18
19 There was no also significant correlation between T level respectively with mean
20 CCs (Fig. 3D, linear regression, $F(1,50) = 0.414, R = 0.091, P = 0.523$) and % of residuals
21 (unclustered sounds) (Fig. 3E, $F(1,50) = 0.23, R = 0.068, P = 0.634$). This suggests that
22 birds with low levels of T at the end of the experiment did not have more plastic songs
23 than birds with high levels of T.

1 3.2. Recordings after transfer to the aviary

2
3 We observed some changes in the acoustics of the songs produced by the finches
4 trained with the various methods. If these changes were subtle in some birds (Fig. 4A and
5 4B), some were more pronounced in others (Fig. 4C and 4D).

6
7 There was a significant positive correlation between overall similarity score at
8 dph 100 and self-similarity score (Fig. 5A, intra-individual comparison between song
9 motif produced at dph 100 and song motif produced later, linear regression, $F(1,40)=$
10 59.246 , $R= 0.773$, $P < 0.001$). This indicates that finches that did not provide a good
11 imitation of the song model were more likely to change their song motif after dph 100
12 than birds that did provide a good imitation. There was no effect of training procedure on
13 this result (general linear model, $F(2,41) = 1.687$, $P = 0.199$).

14 Moreover, we found a significant negative correlation between self-similarity
15 score and % of residuals after data clustering, indicating that birds that changed their
16 song more after training were also those that produced more variable sounds that could
17 not be clustered as distinct syllable types (Fig. 5B, linear regression, $F(1,40) = 19.315$, $R =$
18 0.571 , $P < 0.001$). There was no significant difference between groups of birds trained with
19 different methods (general linear model, $F(2,41) = 0.614$, $P = 0.547$).

20
21 This could also be predicted at the end of the training phase since there was a
22 significant negative correlation between similarity scores at dph 100 and residuals after
23 clustering, meaning that birds that did provide a good imitation of the song model tended

1 to produce more distinct (clustered) syllables than worse learners (Fig. 5C, linear
2 regression, $F(1,58)= 12.459$, $R= 0.421$, $p= 0.001$). There was no significant difference
3 between groups of birds trained with different methods (general linear model, $F(2,59)=$
4 1.364 , $P= 0.264$).

5

6 Regarding song syntax stereotypy, we observed a significant positive correlation
7 between self-similarity score (intra-individual comparison between song motif produced
8 at 100dph and song motif produced later) and the mean of CCs (Fig. 5D, linear
9 regression, $F(1,40)= 33.946$, $R= 0.678$, $P< 0.0001$). This indicates that finches that did
10 not produce stereotyped sequences of song syllables at dph 100 were more likely to
11 change their song after training than birds that did provide a good imitation. There was no
12 effect of training procedure on this result (general linear model, $F(2,41)= 0.690$, $P=$
13 0.508).

14

15 This could also be predicted at the end of the training phase since there was a
16 significant positive correlation between similarity score at dph 100 and mean CCs,
17 meaning that birds that did provide a good imitation of the song model tended to produce
18 more syntax-stereotyped songs than worse learners (Fig. 5E, linear regression, $F(1,58)=$
19 20.869 , $R= 0.514$, $P< 0.0001$). There was no significant difference between groups of
20 birds trained with different methods (general linear model, $F(2,59)= 1.155$, $P= 0.323$).

21

22

23

1 **4. Discussion**

2

3 These results indicate that young male zebra finches learn poorly from passive
4 tape playback even when timing and quota of song exposure are controlled. Better results
5 are obtained when the bird can self-elicite tape playback using an operant conditioning
6 procedure, despite a high inter-individual variability. One-to-one live tutoring is clearly
7 the most effective method to get a close-to-perfect imitation of a song model. Moreover,
8 the less the bird learned during the training period, the more likely it was to transform its
9 song later when raised with conspecifics, highlighting again the importance of social
10 factors on song development. Range of vocal changes could also be predicted at the end
11 of the training period using different criteria to measure song stereotypy, but could not be
12 predicted based solely on testosterone levels.

13

14 *4.1. Vocal changes after training*

15

16 The fact that live tutoring might extend sensitive periods beyond the limits
17 defined by auditory stimulation alone is not new (Baptista and Petrinovitch, 1984, 1986)
18 even in an age-limited learner such as the zebra finch (Morrison and Nottebohm, 1993;
19 Jones et al., 1996).

20 We observed that birds that did provide worse imitation of the song model were
21 those that produced more unclustered sounds and were more likely to change their song
22 as adults. This suggests that bad learning is not a consequence of a lack of plasticity but
23 rather by a lack of an adequate song model provided during the sensitive phase. It does

1 not necessarily mean that the method was inappropriate since even birds trained with a
2 live tutor were likely to change their song when imitation was not fairly complete. We
3 did not observe correlations between the different criteria of song stereotypy and
4 testosterone level. In a previous experiment, Livingston and colleagues (2000) showed
5 that isolated housing conditions delay testosterone increase during maturation, but that
6 differences with normally raised conspecifics disappear in adults. In our study, it would
7 have been interesting to look at the individual time course of testosterone levels.

8 The nature of the vocal changes after training is not clear: the finches might have
9 learned from conspecifics but they might also have invented/improvised new sounds
10 based on those produced before or after dph 100. More experiments are required to
11 explore these aspects. Nevertheless, it has been observed in other studies that some of
12 these vocal changes were syllables learned from conspecifics (Morrison and Nottebohm,
13 1993; Jones et al., 1996).

14 Overall, vocal changes after the so-called sensitive period in birds trained with
15 tapes permitted Baptista and Petrinovitch (1984) to emphasize that live models can be
16 more effective than playbacks.

17

18 *4.2. Passive playback*

19

20 Finches trained with passive playback exhibited the worst learning scores. Passive
21 playback has long been thought to be inefficient in zebra finches but both timing and
22 quota of song exposure were not always controlled accurately. In particular, it was shown
23 that overexposure to taped playback using the operant conditioning procedure could

1 impair song learning (Tchernichovski et al., 1999). Our results suggest that the
2 inefficiency of passive playback is neither due to timing exposure, nor to sound quota.
3 Nevertheless, some learning occurred and there was also some inter-individual
4 variability. The fact that birds trained with playback just before the night exhibited song
5 learning indicate that immediate practice is not necessary in this species where the
6 sensory phase and the sensorimotor phase completely overlap (Roper and Zann, 1996). In
7 a recent experiment, Gehrold, Leitner, Laucht and Derégnaucourt (submitted) succeeded
8 in training young male zebra finches, kept in the same housing conditions as in this study,
9 to imitate short parts of a canary song bout. This suggests that some learning can also
10 occur when the young male zebra finch cannot interact with its environment either
11 through a reward obtained in self-eliciting song playback or through live interactions with
12 a conspecific.

13

14 *4.3. Inaccuracies in song imitation*

15

16 Innaccuracies in song imitation observed in the laboratory might have different
17 causes that are not mutually exclusive (Derégnaucourt, 2011): (1) the methods used to
18 train the bird to imitate a song may not be appropriate; (2) the bird might have
19 memorized the song model but will not produce it; (3) it does not make sense,
20 biologically speaking, to provide a fairly complete imitation. In the zebra finch (and other
21 oscine species), studies have confirmed hypotheses (1) and (2). For example, it has been
22 shown that both good learners and bad learners exhibit a preference for the song model
23 they have been exposed to during the sensitive period (Adret, 1993; Houx and ten Cate,

1 1999a). Tutor song preference can still be observed one year after the birds have been
2 separated from their tutor (Gobes and Bolhuis, 2007). Males could learn the complete
3 tutor song but not incorporate all of the tutor song elements into their own song (Nelson
4 and Marler, 1994; Geberzahn et al., 2002). This assumption was rejected by Terpstra and
5 colleagues (1994) since they observed a positive correlation between gene ZENK
6 expression in NCM (an auditory area) and quality of imitation of the tutor song following
7 exposure to the tutor song in adult finches. Also, inaccuracies in song imitation might
8 facilitate individual recognition (Böhner, 1983). Thus inaccuracies in song imitation
9 should not always be considered as errors or deficits since they could be adaptive and
10 even play a key role in the development of song cultures.

11

12 *4.4. Live tutoring*

13

14 In the starling (*Sturnus vulgaris*), passive playback enables vocal learning by
15 isolated birds whereas learning is weak or inefficient when they are housed with a
16 conspecific companion (Poirier et al., 2004). This might explain why passive playback
17 was initially thought to be inefficient in the zebra finch (Eales, 1989; Immelmann, 1969),
18 but our results show that accurate imitation of a song model cannot be obtained using
19 passive playback in singly isolated finches. Looking at the ultimate aspects of song
20 learning might help to understand why passive playback is not effective and why social
21 factors are so important in the zebra finch. In this species the strong resemblance between
22 tutor and pupil song suggests that for a young bird it is important to produce accurate
23 tutor learning rather than to obtain longer songs or larger repertoires (Gil et al., 2006).

1 Even if song production is used during courtship displays to females (Riebel, 2009), there
2 is no evidence that female zebra finches have a preference for particular syllables, as
3 shown for example in canaries (Vallet and Kreutzer, 1995). Song is not used in a
4 territorial context (Zann, 1996). One possible function is that song could constitute a
5 group signature. Like zebra finches, starlings live in large social groups and also learn
6 better from a live tutor than from taped songs (Chaiken et al., 1993). In this species it was
7 clearly demonstrated that song sharing reflects social affinities (Eens et al., 1992;
8 Hausberger, 1997). These aspects need to be studied in zebra finches.

9

10 *4.5. Developing new methods to train young finches to learn a song*

11

12 Even if fairly complete imitation observed in the one-to-one tutoring is an artefact
13 of captivity, it constitutes a baseline for researchers that use birdsong as a biological
14 model to study the molecular, cellular and behavioural correlates of vocal learning. For
15 example, using this paradigm, Haesler and colleagues (2007) found evidence the role of
16 gene FoxP2 for song learning, establishing birdsong as an interesting model for speech
17 dyspraxia. The problem with live tutoring is that one cannot control the behaviour of the
18 tutor. The association of song with a stuffed model (Bolhuis et al., 1999; Houx and ten
19 Cate, 1999b) or a non-social visual stimulus (Hultsch et al., 1999) confirms the
20 importance of visual and/or auditory components that has been indicated in earlier studies
21 (Todt et al., 1979; Eales, 1989) and that could be involved in a process of associative
22 learning. Since social interactions also appear predominant in the song learning process
23 of the zebra finch, new methods should also take into account these aspects. There is a

1 growing interest for the use of robotics in animal behaviour research (Webb, 2008).
2 Developing a robot zebra finch could allow us to identify the respective salience of each
3 stimulus for song acquisition.

4

5 **5. References**

6

7 Adret, P. (1993). Operant conditioning, song learning and imprinting to taped song in the
8 zebra finch. *Anim. Behav.* 46, 149-159.

9 Arnold, A. P. (1975a). The effects of castration on song development in zebra finches
10 (*Poephila guttata*). *J. Exp. Zool.* 191, 261-278.

11 Arnold, A.P. (1975b). The effects of castration and androgen replacement on song,
12 courtship, and aggression in zebra finches (*Poephila guttata*). *J. Exp. Zool.* 191,309-326.

13 Baptista, L. F., and Gaunt, S. L. L. (1997). Social interaction and vocal development in
14 birds. In *Social influences on vocal development*, C.T. Snowdon and M. Hausberger, ed.
15 (Cambridge: Cambridge University Press), pp. 23-40.

16 Baptista, L. F., and Petrinovitch, L. (1984). Social interaction, sensitive phases and the
17 song template hypothesis in the white-crowned Sparrow? *Anim. Behav.* 32, 172-181.

18 Baptista, L. F., and Petrinovitch, L. (1986). Song development in the white-crowned
19 sparrow: social factors and sex differences. *Anim. Behav.* 34, 1359-1371.

20 Böhner, J. (1983). Song learning in the zebra finch (*Taeniopygia guttata*): selectivity in
21 the choice of a tutor and accuracy of song copies. *Anim. Behav.* 31, 231-237.

22 Bolhuis, J. J., Van Mil, D. P., and Houx, B. B. (1999). Song learning with audiovisual
23 compound stimuli in zebra finches. *Anim. Behav.* 58, 1285-1292.

1 Bottjer, S. W. and Hewer, S. J. (1992). Castration and antisteroid treatment impair vocal
2 learning in male zebra finches. *J. Neurobiol.* 23, 337-353.

3 Brainard, M. S., and Doupe, A. J. (2002). What songbirds teach us about learning. *Nature*
4 417, 351-358.

5 Brenowitz, E. A., Lent, K. L., and Kroodsma, D. E. (1995). Brain space for learned song
6 in birds develop independently of song learning. *J. Neurosci.* 15, 6281-6286.

7 Catchpole, C. K. and Slater, P. J. B. (2008) *Bird song: biological themes and variations.*
8 2nd ed. (Cambridge: Cambridge University Press).

9 Chaiken, M., Böhner, J., and Marler, P. (1993). Song acquisition in European starlings: a
10 comparison of the songs of live-tutored, tape-tutored, untutored, and wild-caught males.
11 *Anim. Behav.* 46, 1079-1090.

12 Derégnaucourt, S. (2011). Birdsong learning in the laboratory, with especial reference to
13 the song of the zebra finch (*Taeniopygia guttata*). *Interact. Stud.* 12, 323-349.

14 Derégnaucourt, S., Mitra, P. P., Fehér, O., Pytte, C., and Tchernichovski, O. (2005). How
15 sleep affects the developmental learning of bird song. *Nature* 433, 710-716.

16 Eales, L. A. (1985). Song learning in zebra finches: some effects of song model
17 availability on what is learnt and when. *Anim. Behav.* 33, 1293-1300.

18 Eales, L. A. (1989). The influences of visual and vocal interaction on song learning in
19 zebra finches. *Anim. Behav.* 37, 507-520.

20 Eens, M., Pinxten, R., and Verheyen, R. F. (1992). Song learning in captive European
21 starlings, *Sturnus vulgaris*. *Anim. Behav.* 44, 1131-1143.

22 Funabiki, Y., and Funabiki, K. (2009). Factors limiting song acquisition in adult zebra
23 finches. *Dev. Neurobiol.* 69, 752-759.

1 Geberzahn, N., Hultsch, H. and Todt, D. (2002). Latent song type memories are
2 accessible through auditory stimulation in a hand-reared songbird. *Anim. Behav.* 64, 783-
3 790.

4 Gil, D., Naguib, M., Riebel, K., Rutstein, A., and Gahr, M. (2006). Early condition, song
5 learning, and the volume of song brain nuclei in the zebra finch (*Taeniopygia guttata*). *J.*
6 *Neurobiol.* 66, 1602-1612.

7 Gobes, S. M. H., and Bolhuis, J. J. (2007). Birdsong memory: a neural dissociation
8 between song recognition and production. *Curr. Biol.* 17, 1-5.

9 Goymann, W., Geue, D., Schwabl, I., Flinks, H., Schmidl, D., Schwabl, H., and Gwinner,
10 E. (2006) Testosterone and corticosterone during the breeding cycle of equatorial and
11 European stonechats (*Saxicola torquata axillaris* and *S. t. rubicola*). *Horm. Behav.* 50,
12 779-785.

13 Haesler, S., Rochefort, C., Georgi, B., Licznarski, P., Osten, P., and Scharff, C. (2007).
14 Incomplete and inaccurate vocal imitation after knockdown of FoxP2 in songbird basal
15 ganglia nucleus area X. *PLoS Biol.* 5, e321.

16 Hausberger, M. (1997). Social influences on song acquisition and sharing in the
17 European starling (*Sturnus vulgaris*). In *Social influences on vocal development*, C. T.
18 Snowdon and M. Hausberger, ed. (Cambridge: Cambridge University Press), pp. 128-
19 156.

20 Hausberger, M., Richard-Yris, M.-A., Henry, L., Lepage, L., and Schmidt, I. (1995).
21 Song sharing reflects the social organization in a captive group of European starlings
22 (*Sturnus vulgaris*). *J. Comp. Psychol.* 109, 222-241.

1 Houx, B. B., and ten Cate, C. (1999a) Song learning from playback in zebra finches: is
2 there an effect of operant contingency. *Anim. Behav.* 57, 837-845.

3 Houx, B. B., and ten Cate, C. (1999b). Do stimulus-stimulus contingencies affect song
4 learning in zebra finches (*Taeniopygia guttata*). *J. Comp. Psychol.* 113, 235-242.

5 Houx, B. B., ten Cate, C., and Feuth, E. (2000). Variations in zebra finch song copying:
6 an examination of the relationship with tutor song quality and pupil behaviour. *Behav.*
7 137, 1377-1389.

8 Hultsch, H., and Todt, D. (1989). Song acquisition and acquisition constraints in the
9 nightingale, *Luscinia megarhynchos*. *Naturwissenschaften* 76, 83–85.

10 Hultsch, H., Schleuss, F. and Todt, D. (1999). Auditory-visual stimulus pairing enhances
11 perceptual learning in a songbird. *Anim. Behav.* 58, 143-149.

12 Immelmann, K. (1969). Song development in the zebra finch and other estrildid finches.
13 In *Bird vocalizations*, R.A. Hinde, ed. (Cambridge: University Press), pp. 61-74.

14 Jones, A. E., Slater, P. J. B., and ten Cate, C. (1996). Early experience and plasticity in
15 adult male zebra finches (*Taeniopygia guttata*). *J. Comp. Psychol.* 110, 354-369.

16 Kojima, S., and Doupe, A. J. (2011). Social performance reveals unexpected vocal
17 competency in young songbirds. *Proc. Natl. Acad. Sci. USA* 108, 1687-1692.

18 Korsia, S. and Bottjer, S. W. (1991). Chronic testosterone treatment impairs vocal
19 learning in male zebra finches during a restricted period of development. *J. Neurosci.* 11,
20 2362-2371.

21 Kroodsma, D. E., and Pickert, R. (1980). Environmentally dependent sensitive periods
22 for avian vocal learning. *Nature* 288, 477-479.

1 Livingston, F. S., White, S. A., and Mooney, R. (2000). Slow NMDA-EPSCs at synapses
2 critical for song development are not required for song learning in zebra finches. *Nature*
3 *Neurosci.* 3, 482-488.

4 Mann, N. I., and Slater, P. J. B. (1995). Song tutor choice by zebra finches in aviaries.
5 *Anim. Behav.* 49, 811-820.

6 Marler, P. (1970). A comparative approach to vocal learning: song development in white-
7 crowned sparrows. *J. Comp. Physiol. Psychol.* 71, 1-25.

8 Marler, P., and Peters, S. (1987). A sensitive period for song acquisition in the song
9 sparrow, *Melospiza melodia*: a case of age-limited learning. *Ethology* 76, 89-100.

10 Marler, P., and Peters, S. (1988). Sensitive periods for song acquisition from tape
11 recordings and live tutors in the swamp sparrow, *Melospiza georgiana*. *Ethology* 77, 76-
12 84.

13 Marler, P., Peters, S., and Wingfield, J. (1987). Correlations between song acquisition,
14 song production, and plasma levels of testosterone and estradiol in sparrows. *J.*
15 *Neurobiol.* 18, 531-548.

16 Marler, P., Mundinger, P., Waser, M. S., and Lutjen, A. (1972). Effects of acoustical
17 stimulation and deprivation on song development in red-winged blackbirds (*Agelaius*
18 *phoeniceus*). *Anim. Behav.* 20, 586-606.

19 Morrison, R. G., and Nottebohm, F. (1993). Role of a telencephalic nucleus in the
20 delayed song learning of socially isolated zebra finches. *J. Neurobiol.* 24, 1045-1064.

21 Mundinger, P. C. (1995). Behaviour-genetic analysis of canary song: inter-strain
22 differences in sensory learning, and epigenetic rules. *Anim. Behav.* 50, 1491-1511.

1 Nelson, D. A. and Marler, P. (1994). Selection-based learning in bird song development.
2 Proc. Natl Acad. Sci. USA 91, 10498-10501.

3 Nicolai, J. (1959). Familientradition in der Gesangsentwicklung des Gimpels (*Pyrrhula*
4 *pyrrhula*). J. Ornithol., 100: 39-46.

5 Peters, S., Marler, P., and Nowicki, S. (1992). Song sparrows learn from limited exposure
6 to song models. Condor 94, 1016-1019.

7 Petrinovitch, L. (1985). Factors influencing song development in the White-crowned
8 Sparrow (*Zonotrichia leucophrys*). J. Comp. Psychol. 99, 15-29.

9 Poirier, C., Henry, L., Mathelier, M., Lumineau, S., Cousillas, H., and Hausberger, M.
10 (2004). Direct social contacts override auditory information in the song-learning process
11 in Starlings (*Sturnus vulgaris*). J. Comp. Psychol. 118, 179-193.

12 Price, P. H. (1979). Developmental determinants of structure in zebra finch song. J.
13 Comp. Physiol. Psychol. 93, 260-277.

14 Pröve, E. (1974). Der Einfluss von Kastration und Testosteronsubstitution auf das
15 Sexualverhalten männlicher Zebrafinken (*Taeniopygia guttata castanotis* Gould). J.
16 Ornithol. 115, 338-347.

17 Pröve, E. (1981). Der Einfluss sozialer Haltungsbedingungen auf die Balzaktivität und
18 Androgentiter männlicher Zebrafinken (*Taeniopygia guttata castanotis* Gould).
19 Verhandlungen der Deutsch. Zool. Gesellschaft 74, 255.

20 Pröve, E. (1983). Hormonal correlates of behavioural development in male zebra finches.
21 In Hormones and behaviour in higher vertebrates, J. Balthazart, E. Pröve and R. Gilles,
22 ed. (Berlin/Heidelberg: Springer-Verlag), pp. 368-374.

1 Riebel, K. (2009). Song and female choice in zebra finches: a review. *Adv. Stud. Behav.*
2 40, 197-238.

3 Roper, A., and Zann, R. (2006). The onset of song learning and song tutor selection in
4 fledging zebra finches. *Ethology* 112, 458-470.

5 Scharff, C., and Nottebohm, F. (1991). A comparative study of the behavioral deficits
6 following lesions of various parts of the zebra finch song system: implications for vocal
7 learning. *J. Neurosci.* 11, 2896-2913.

8 Tchernichovski, O., Lints, T., Mitra, P. P., and Nottebohm, F. (1999). Vocal imitation in
9 zebra finches is inversely related to model abundance. *Proc. Natl Acad. Sci. USA* 96,
10 12901-12904.

11 Tchernichovski, O., Nottebohm, F., Ho, C. E., Pesaran, B. and Mitra, P. P. (2000). A
12 procedure for an automated measurement of song similarity. *Anim. Behav.* 59, 1167-
13 1176.

14 Tchernichovski, O., Mitra, P. P., Lints T. J., and Nottebohm, F. (2001). Dynamics of the
15 vocal imitation process: how a zebra finch learns its song. *Science* 291, 2564-2569.

16 Tchernichovski, O., Lints, T. J., Derégnaucourt, S., Cimenser, A., and Mitra, P. P. (2004).
17 Studying the song development process: rationale and methods. *Ann. NY Acad. Sci.*
18 1016, 348-363.

19 Terpstra, N. J., Bolhuis, J.J., and den Boer-Visser, A.M. (2004). An analysis of the neural
20 representation of birdsong memory. *J. Neurosci.* 24, 4971-4977.

21 Thielke, G. (1970). Lernen von Gesang als möglicher Schrittmacher der Evolution.
22 *Zeitschrift für Zoologische Systematik und Evolutionforschung*, 8, 309–320.

- 1 Thorpe, W. H. (1954). The process of song learning in the Chaffinch as studied by means
2 of the sound spectrograph. *Nature* 173, 465-469.
- 3 Thorpe, W. H. (1958). The learning of song patterns by birds, with especial reference to
4 the song of the chaffinch *Fringilla coelebs*. *Ibis* 100, 535-570.
- 5 Todt, D., Hultsch, H., and Heike, D. (1979). Conditions affecting song acquisition in
6 Nightingales (*Luscinia megarhynchos L.*). *Z. Tierpsychol.* 51, 23-35.
- 7 Vallet, E., and Kreutzer, M. (1995). Female canaries are sexually responsive to special
8 song phrases. *Anim. Behav.* 49, 1603-1610.
- 9 Webb, B. (2008) Using robots to understand animal behavior. *Adv. Stud. Behav.* 38, 1-
10 58.
- 11 Williams, H., Connor, D. M., and Hill, J. W. (2003). Testosterone decreases the potential
12 for song plasticity in adult male zebra finches. *Horm. Behav.* 44, 402-412.
- 13 Zann, R. (1996). *The zebra finch: a synthesis of field and laboratory studies* (Oxford:
14 Oxford University Press).
- 15 Zeigler, H.P., and Marler, P. (2008). *Neuroscience of birdsong* (Cambridge: Cambridge
16 University Press).
- 17

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2

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8

9 **Legends of figures**

10

11 Fig. 1. Spectrograms of songs produced by adult male zebra finches. A to C: three song
12 motifs produced by three different males. These songs were used as song models for the
13 experiment. D: song of the best learner from the live tutor group. E: song of the best
14 learner from the operant training group. F: song of the best learner from the passive
15 playback group.

16

17 Fig. 2. Comparison of the different methods used to train a young zebra finch to learn a
18 song. A: similarity, accuracy and sequential match to the song model for finches trained
19 with the three different methods: live tutoring, operant conditioning and passive
20 playback. B: similarity, accuracy and sequential match to the song model for two groups
21 of birds trained with operant conditioning: one daily session of 20 key pecks (starting in
22 the morning) and two daily sessions of 10 key pecks each (one in the morning and one in
23 the afternoon). C: similarity, accuracy and sequential match to the song model for two

1 groups of birds trained with passive playback: one exposure of 20 song models in the
2 morning starting 1 min after lights on, and one exposure of 20 song models in the
3 evening ending 1 min before lights off.

4

5 Fig. 3. Singing activity, testosterone level and song stereotypy at the end of the training
6 phase. A: correlation between overall similarity scores and singing activity. B: correlation
7 between overall similarity score and testosterone level. C: correlation between singing
8 activity and testosterone level. D: correlation between song syntax stereotypy (mean
9 CCs) and testosterone level. E: correlation between unclustered sounds and testosterone
10 level. See the text for details about the acoustic measures.

11

12 Fig. 4. Spectrograms of songs produced by adult male zebra finches. A and B: two
13 exemplars of songs produced by the same male at dph 100 (A) and several months later
14 after transfer to aviary. In the case of this bird, changes in the acoustic structure of the
15 song were subtle. C and D: songs of the bird that exhibited the most changes in its song
16 following transfer to aviary after 100 dph. C: song at dph 100; D: song recorded several
17 months later.

18

19 Fig. 5. Changes in the acoustic structure of the song following transfer to aviary after 100
20 dph. A: correlation between overall similarity score at dph 100 and self-similarity score.
21 B: correlation between unclustered sounds at dph 100 and self-similarity score. C:
22 correlation between unclustered sounds at dph 100 and overall similarity score at 100
23 dph. D: correlation between mean CCs at dph 100 and self-similarity score. E: correlation

- 1 between mean CCs at dph 100 and self-similarity score. See the text for details about the
- 2 acoustic measures.
- 3