Zebra finches go wild! Experimental cultural evolution of birdsong.

Adriana Diez ^{1,2, *} and Scott A. MacDougall-Shackleton ^{2,3}

¹ Graduate Program in Neuroscience, University of Western Ontario, London, ON, Canada

² Advanced Facility for Avian Research, University of Western Ontario, London, ON, Canada

³ Department of Psychology, University of Western Ontario, London, ON, Canada

Author contributions: A.D.: conducted all experimental work

A.D., S.M-S.: design, data analysis and manuscript preparation.

Correspondence to: MacDougall-Shackleton, S. Email: smacdou2@uwo.ca.

Summary

Vocal learning in songbirds is guided by experience and experience-independent factors. Previously, lineages of zebra finches founded by isolate-reared tutors showed cultural evolution to wild-type song features. This suggests that biases lead to selective imitation even in the absence of wild-type song. We hypothesized that cultural evolution of song depends on both experience-independent biases and the tutor songs available. We predicted that songs more distant from wild-type would take longer to culturally evolve toward wild-type features. We bred zebra finches in three groups of lineages in which offspring of each generation served as tutors for the next. Lineages were founded with males singing wild-type song, isolate song, or heterospecific song. The two experimental lineages exhibited rapid cultural evolution of song with many features converging to wild-type within two generations. However the rate of change differed depending on song features measured, and took longer for lineages founded with heterospecific song.

Keywords: birdsong; song learning; zebra finch; Bengalese finch; cultural evolution

INTRODUCTION

Birdsong is one of the most extensively studied forms of animal communication and has become an important model to study imitative vocal learning. Unlike language, imitative vocal learning is not unique to humans and is widespread among birds (Jarvis, 2007; Bolhuis et al., 2010). Birdsong learning and speech development share many features including progressive development of vocalizations, the influence of social interactions, and a critical role of auditory feedback (Doupe & Kuhl, 1999). Songbirds have, therefore, become a common behavioral and neural model to study vocal learning as a perceptual and motor ability (Brainard & Doupe, 2002).

Birdsong development is guided by multiple experiential factors including exposure to tutor songs and social interactions (Baptista & Petrinovich, 1986; Houx & Ten Cate, 1998; Beecher & Brenowitz, 2005; Soma, 2011; Ljubicic et al., 2016; Beecher, 2017). Songbirds raised in captivity will typically learn the song of conspecifics to which they are exposed. In some cases birds will even learn the songs of heterospecific tutors (Immelmann, 1972; Baptista & Morton, 1981; Baptista & Petrinovich, 1984; Clayton, 1989). Additionally, social feedback from females shapes the development of male song in cowbirds (*Molothrus ater*) and zebra finches (*Taeniopygia guttata*) (e.g., King & West, 1983; West & King, 1988; Carouso-Peck & Goldstein, 2019). The effects of social experience on song development extend beyond interactions with female receivers, and include social interactions amongst young peers during the song learning process (Chaiken et al., 1997; Poirer et al., 2004)

In addition to social experience, the experience of hearing one's own vocalizations - auditory feedback- is critical to song development. For example, seminal experiments on deafened white-crowned sparrows (*Zonotrichia leucophrys nuttalli*) and Mexican juncos (*Junco phaeonotus*) showed that the songs produced by deafened birds lacked both fundamental species-specific and dialectal features (Konishi, 1964, 1965). The fact that songs of deafened birds have fewer species-typical features indicates the importance of auditory feedback. In addition to experience with tutor songs and auditory feedback, developmental experience in general can affect song learning (Spencer & MacDougall-Shackleton, 2011; MacDougall-Shackleton & Spencer, 2012; Peters et al., 2014).

Beyond the critical role of experience in birdsong development, experience-independent factors also guide song development in many species. Shortly after the discovery of the

importance of experience with a tutor song for song learning, it also became clear that songbirds have predispositions to learn species-typical sounds. Young swamp sparrows (*Melospiza georgiana*) learned only conspecific syllables when tutored with both swamp sparrow and song sparrow (*M. melodia*) syllables (Marler 1977). Furthermore, the songs of isolate-reared birds contain many species-typical features, indicating experience-independent predisposition to produce certain vocalizations (Thorpe, 1954; Konishi, 1965; Price, 1979; Williams et al., 1993; Lachlan & Slater 1999; Marler & Zeigler, 2008). Despite these experience-independent biases, many species of songbird will indeed learn to imitate heterospecific sounds if conspecific tutors are not available (e.g., Baptista & Petrinovich, 1984), and some species routinely learn heterospecific song through vocal mimicry (Dalziell et al., 2014). Thus, song development depends on an interaction between social and auditory experience and experience-independent biases.

Birdsong development is thus a clear example of gene-culture interaction (Lachlan & Slater, 1999). Imitative vocal learning is produced by an interaction between genes that bias the recognition and learning of conspecific song features, as well as the culturally transmitted songs themselves and social interactions (Lachlan & Slater, 1999; Lachlan & Feldman, 2003; Lachlan, Janik, & Slater, 2004; Whiten et al., 2017). Social interactions can augment experience-independent biases in song development. For example, pair-housed starlings (*Sturnus vulgaris*) with no adult tutors developed songs with more species-typical features than individually isolated starlings (Chaiken et al., 1997). However, experience-independent biases affect which song features are learned from a social tutor. Wild-type zebra finch (*Taeniopygia guttata*) song features emerged de novo within a few generations in lineages of finches founded by birds singing isolate songs (Fehér et al., 2009). Young zebra finches exposed to this abnormal song, through imitation and accumulation of changes over generations, modified the original isolate song towards a song with wild-type features, showing learning biased towards wild-type zebra finch features. However, in the Fehér et al. (2009) study it was not determined if the rapidity of the cultural evolution over generations depended on how similar the initial tutor song was to the tutees' experience-independent biases.

In this study our objective was to explore the interaction of i) experience-independent biases in song learning and ii) the nature of the tutor song in the cultural evolution of song across generations. Building on the study by Fehér et al., (2009), we hypothesized that cultural

evolution towards species-typical song features should be more rapid if the song of the founding generation has more species-typical features. To test this, we created three groups of social lineages founded by song tutors singing wild-type zebra finch song (control group), isolate song, or heterospecific song. We predicted that, over generations, the isolate-tutored song lineages should more rapidly evolve toward wild-type song features than the heterospecific-tutored lineages, as the latter contains fewer species-typical song features.

METHODS

Study subjects and general timeline

All the experimental procedures, care, and housing conditions followed the guidelines of the Canadian Council on Animal Care and were approved by the University of Western Ontario's Animal Use Subcommittee.

Male zebra finches used in this experiment were bred from a colony maintained at the Advanced Facility for Avian Research (AFAR) at the University of Western Ontario. We paired males and females in individual breeding cages with a nest cup and *ad libitum* access to multi-vitamin seeds, cuttlefish bone, grit, and water. During the breeding period, we provided daily, one tablespoon (approx. 15 g) of egg-food mix (blended hard-boiled egg and bread). Once the chicks hatched we increased the amount of egg food mix to one tablespoon per chick until age of 45 (± 2) days post hatch (dph). During the entire experiment, the rooms were kept at 24 °C and on a 14 h :10 h light:dark photoperiod. The breeding process took over a year and a half, and was synchronized with the song tutoring process.

The breeding pairs produced 85 male zebra finch offspring in 26 clutches. Both parents reared the young until 10 dph of the oldest chick in the clutch. At that day, we moved the offspring and their mothers to a nursery room with other females (who do not sing) and chicks to avoid the offspring being exposed to their father's or other male's song, until 35 (± 2) dph, when the young zebra finches reached feeding independence. Consequently, our protocol should have limited males from hearing or learning their genetic father's song or any male's song (Roper & Zann 2006). Offspring were genetically sexed at about 20 dph, via collection of a small blood sample, DNA extraction and PCR amplification of sex chromosome markers.

Once offspring reached feeding independence (approx. 35 dph) the male offspring (from now on called pupils) were randomly assigned to one of the three song tutoring conditions or

lineages (described below). In general, or tutoring protocol was similar to that of Fehér et al. (2009). In the tutoring conditions, each pupil was individually housed with a mature (+120 dph), unrelated zebra finch male (from now on the tutor) for 90 days. At this point the pupil's song was recorded twice, with 7 days in between, to confirm that the song was crystalized. After their songs were recorded, the pupils were assigned as tutors for a new pupil generation. Thus, after the initial generation of tutors from the different tutoring conditions or lineages, we set up a line of pupils, ('the first generation'). Once the first generation of pupils achieved maturity and crystalized their song, they became tutors for the next generation, ('the second generation'). Once the pupils from the second generation reached maturity and crystalized their songs, they became tutors for the next generation and so on for 4 generations in total. The overall procedure is illustrated in Figure 1.

We used the above procedures to create three song tutoring lineages. Similar to prior a prior study we created an isolate song tutoring lineage (Fehér et al., 2009). In addition, we created two more lineages: a control lineage founded by zebra finches that sing wild-type song, and heterospecific tutored lineage of zebra finches that were initially tutored by Bengalese finches (*Lonchura striata domestica*) (Figure 1).

Heterospecific song lineage

The first generation of subjects in the heterospecific song condition were exposed to wild-type heterospecific songs from a baseline (tutor group). To obtain the baseline tutor group we used six adult male Bengalese finches. We chose Bengalese finches as heterospecific tutors because male zebra finches tutored by Bengalese finches are able to imitate Bengalese finch song (Clayton, 1987). Also, Bengalese finch song is different enough to the zebra finch song that we are able to easily identify them (Figure 2). Finally, Bengalese finch syllable types and syllable syntax rarely appear in wild-type zebra finch songs (Funabiki & Konishi, 2003). To found this lineage we collected fertilized zebra finch eggs from the colony and transferred them to nests of Bengalese finches, who incubated the zebra finch eggs and raised the offspring.

Isolate song lineage

The first generation of subjects in the isolate song condition were exposed to isolate zebra finch songs. To obtain the baseline tutor group we used seven male zebra finches from the zebra finch colony. The breeding conditions to produce these males were the same as described above, however, when the young males reached independence at age 35 (± 2) dph, they were

individually housed in a soundproof attenuation chamber until age 140 dph. An isolate song develops when males are kept in such social and acoustic isolation throughout their song-learning period (day 10-90; Williams et al., 1993). An example of the isolate song produced by these isolate song tutors is illustrated in Figure 2.

Wild-type conspecific song lineage

The first generation of subjects in the wild-type conspecific condition were exposed to wild-type zebra finch song from a baseline (tutor group) over three generations. To obtain the baseline tutor group we used seven adult male zebra finches from our zebra finch breeding colony that all sang a wild-type song (Figure 2).

Song recording

We recorded female-directed songs from all the tutors and pupils from the three tutoring lineages described above (heterospecific song, isolate song, wild-type song). The songs were recorded after song crystallization at the age of 120 dph or older. To obtain the song recording, each male was kept in isolation in a sound attenuation chamber for around 20 h. Following this isolation period, a conspecific female was placed in an adjacent cage in the chamber to motivate the male to sing. These directed songs were obtained from two recording sessions of 10 min. We had an interval of seven days between each session to guarantee song crystallization. Songs were digitally recorded using an omni-directional microphone (Sennheiser ME62/K6P) and a digital audio recorder (MARANTZ PMD671) with a sampling rate of 44.1 kHz and 32-bit per second.

Song analysis

The song recordings were segmented in song-bouts and song-units (Figure 2) using Raven Pro software (version 1.4, Cornell Lab of Ornithology). This segmentation allowed us to create a folder that contained files with the song-bouts produced by each bird. From these files, we selected the longest song-bout that contained the highest number of song-units. Then we subdivided the song-units and created a folder that contained files with the song-units found in that song-bout. Following Holveck et al., (2008) the longest and most complete song-bouts and song-units were selected for analyses.

The above-described segmentation allowed us to analyze the songs at three levels: song-bouts, song-units and syllables, for further comparisons between lineages and generations (Figure 2). For the purposes of this song analysis, any introductory syllables were not included

in the measurements. To obtain all the quantitative measurements for the three levels (song-bouts, song-units and syllables) we used Sound Analysis Pro (SAP) 2011 (version 2011.107; freely available at: <http://soundanalysispro.com>, for more information see (Tchernichovski et al., (2000). SAP automatically extracted measurements of mean, minima, maxima and variances for 9 different traits: amplitude, pitch, mean frequency, goodness of pitch, AM, FM, Wiener entropy, continuity over time (t), continuity over frequency (f) (Table 1). We included the measurement of mean, minima and maxima values for every song trait in the analysis, because the three types of songs (heterospecific, isolate, and wild-type conspecific) show differences in these parameters.

To obtain the song-bout and song-unit spectral measurements, we manually selected the start and end of every song-bout and song-unit, and SAP automatically calculated the variable. To calculate the syllable spectral measurements, we opened the song-unit selected for analysis and SAP segmented the syllables automatically. For the analysis, we used the default parameters defined by SAP (40 dB threshold for amplitude and 264 threshold for pitch) for the song-bouts and song-unit analysis, measures of singing performance and song complexity.

Statistics

Our analysis strategy consisted of first performing a linear discriminant function analysis of the initial tutor songs used to found the three tutor song lineages. Then, we classified the songs from new generations based on these discriminant function models. We replicated this approach at three levels of song analysis: 1. coarse-grained model – song-bout analysis, 2. midway-grained model - song-unit analysis, and 3. fine-grained model – syllables analysis.

To conduct the above analyses we used Fisher's linear discriminant function (ldf) analysis implemented by SPSS 21.0 to derive three predictive ldf models based on the three levels of song analysis. Each of these three models were developed from the baseline-tutor group for each lineage (heterospecific song, isolate song, wild-type song). Then, these models were used to classify the new cases from the subsequent generations.

Before we ran the discriminant function analysis, we eliminated the parameters or variables (Table 1) that were redundant for each model. To do this, we developed a correlation matrix for each model that contained all the variables, then we manually removed one of the variables in which the Pearson correlation value was higher than 0.500. After the highly

correlated variables were removed, we ran the discriminant functional models including only the low correlated variables. A further elimination of variables was made by using the stepwise F to enter F to remove procedure; specifically we used the Wilks method with a F of 3.84 as inclusion and F 2.71 for elimination of variables criterion. Therefore, only the parameters considered significant predictor variables were included in the final model.

When the initial tutor discrimination models for the three levels of analysis were obtained, we added the subsequent generations and lineages to assign each case to one of the three tutor groups. To test that the group classification was accurate we used the leave-one-out cross-validation procedure, that sequentially removed each individual out of the calculations to derive the discriminant functions. Subsequently, that individual was classified based on these functions.

RESULTS

We successfully bred a total of 85 male zebra finches. The number of male offspring in each generation in lineage are indicated in Figure 1. Because our sample sizes were small for the third and fourth experimental generation we provide descriptive results for these cohorts only.

Overall, we found that the lineages founded by birds singing isolate song or heterospecific song quickly evolved towards wild-type song features at all levels of song organization (song bout, song unit, syllable). However, the rates of cultural evolution varied across groups and levels of song analysis (Figures 3).

Discriminant function analysis of Tutor Songs:

Coarse-grained features – song-bout analysis. We entered 19 variables in the analysis, and the discriminant function stepwise forward procedure extracted four variables that maximally separated the three tutor groups: *song-bout goodness of pitch*, *song-unit density per second*, *minima amplitude modulation*, and *maxima entropy* (Table 2). The overall Chi-square test was significant (Wilks $\lambda = .083$, Chi-square = 38.529, $df = 8$, Canonical correlation = 0.90, $p < 0.001$). The weighted combination of these predictor variables lead to two canonical functions. The two discriminant functions obtained explained 92% of the variance in song's differentiation between the groups, the function 1 explained 78% and function 2 explained the remaining 22%. The cross-validated classification showed that overall 80% of the grouped cases were correctly classified, contrasted with 42% by chance. Reclassification of cases was based on the original

classification of canonical variables that was highly successful: 90.0 % of the cases were correctly classified into their original categories.

Medium-grained features - song-unit analysis. We entered 18 variables in the analysis, and the discriminant function stepwise forward procedure extracted four that maximally separated the three tutor groups. These predictor variables were: *standard deviation mean frequency, peak frequency, standard deviation amplitude, and frequency modulation* (Table 2). The overall Chi-square test was significant (Wilks $\lambda = 0.075$, Chi-square = 40.085, df = 8, Canonical correlation = 0.93, $p < 0.001$). The weighted combination of these predictor variables lead to two canonical functions explaining 93% of the variance in song-units differentiation between the groups. Cross-validated classification showed that overall 70% of the grouped cases were correctly classified, contrasted with 42% by chance. Reclassification of cases was based on the original classification of canonical variables that was highly successful: 85.0 % of the cases were correctly classified into their original categories.

Fine-grained features – syllables analysis. We entered 41 variables in the analysis, and the discriminant function stepwise forward procedure extracted ten that maximally separate the three tutor groups. These predictor variables were: *Syllable frequency modulation, maxima amplitude, standard deviation amplitude modulation, standard deviation mean frequency, amplitude, goodness of pitch, minimum frequency modulation, maxima goodness of pitch, minima peak frequency, minima spectral continuity over frequency*, (Table 2). The overall Chi-square test was significant (Wilks $\lambda = 0.033$, Chi-square = 268.84, df = 20, Canonical correlation = 0.764, $p < 0.001$). The weighted combination of these predictor variables lead to two canonical functions that explained 67% of the variance in syllable differentiation between the groups. Cross-validated classification showed that overall 78.5 % of the grouped cases were correctly classified, contrasted with 42% by chance. Reclassification of cases was based on the original classification of canonical variables that was successful: 75.6.0 % of the cases were correctly classified into their original categories.

Cultural evolution of song

To characterize changes in song features over generations, we used the discriminant functions generated using the tutor songs (above) to quantify song features of birds from each generation, within each song lineage group (wild-type, isolate, heterospecific). We repeated this

process at each level of song organization (bout, song unit, syllable). In general, song features at all three levels of organization quickly converged to wild-type, consistent with prior findings (Feher et al. 2009). Both the lineages founded with isolate song tutors and heterospecific song tutors rapidly evolved toward songs whose features overlapped almost completely with wild-type song features within two generations (Figure 3). However, the progression of these features differed between levels of song organization and tutor lineage.

Coarse-grained features – song-bout analysis. In the first generation, songs of both the isolate tutor lineage and the heterospecific tutor lineage quickly shared several features with wild-type tutor songs (Figure 4). To quantify this, we calculated the distance of each song from the centroid of the wild-type tutor songs from the tutor generation. A two-way ANOVA including the tutor generation and first two experimental generations indicated that the distance to the wild-type centroid differed between lineage groups ($F_{2,68}=6.4$, $P=0.047$), and that there was a significant interaction between generation number and lineage groups ($F_{4,68}=2.5$, $P=0.003$). Post hoc analyses for each generation demonstrate that in the tutor generation all three groups were significantly different from each other, but that there was no significant difference between lineages in generations 1 or 2 (Figure 5).

We also tested how well our discriminant functions categorized songs to the appropriate lineage. By the first generation the functions accurately assigned all wild-type songs to the wild-type lineage, however only about half of the isolate and heterospecific lineages were correctly categorized (Table 3). Combined with the above results, these data indicate that song bout features quickly evolved toward wild-type with the first generation for both isolate and heterospecific tutored lineages.

Medium-grained features - song-unit analysis. From the first generation, song-units of the isolate tutor lineage shared several features with wild-type tutor songs (Figure 6). In contrast, song-units of the heterospecific tutor lineage took more than one generation to resemble features of wild type song units. We calculated the distance of each song from the centroid of the wild-type tutor generation songs (Figure 7). A two-way ANOVA including the tutor generation and first two experimental generations indicated that the distance to the wild-type centroid differed between lineage groups ($F_{2,69}=39.6$, $P=0.0001$), and that there was a significant interaction between generation number and lineage group ($F_{4,69}=7.1$, $P=0.0001$). Post hoc analyses for each

generation demonstrate that in the tutor generation all three groups were significantly different from each other. In generation 1 the heterospecific lineage differed from both the wild-type and isolate lineages, which did not differ from each other. There were no significant differences between lineages in generation 2 (Figure 7).

We also tested how well our discriminant functions categorized songs units to the appropriate lineage. The categorization of song-units to the wild-type category took one generation in the song-units produced by the isolate lineage and two generations for the song-units produced by the heterospecific lineages (Table 3). Additionally, the heterospecific lineage song-units shared more features with the isolate lineage than with the wild-type lineage by the second generation (Table 3). Combined with the above results, these data indicate that song unit features evolved toward wild-type but it took longer than for song bout features, particularly for the heterospecific lineage. Moreover, the closer resemblance of the song-units of the heterospecific lineage to the isolate lineage compared to the wild-type could indicate that song-units from the heterospecific lineage require to pass through a stage more similar to isolate song bouts before evolving toward wild-type song bout features.

Fine-grained features – syllables analysis. At the syllable level it took until generation 2 until syllables of both the isolate tutor lineage and the heterospecific tutor lineage shared more features with the wild-type tutor lineage syllables (Figure 8). To quantify this, we calculated the distance of each song from the centroid of the wild-type tutor songs from the tutor generation (Figure 9). A two-way ANOVA including the tutor generation and first two experimental generations indicated that the distance to the wild-type centroid differed between lineage groups ($F_{2,813}=75.5$, $P=0.0001$) and across generations ($F_{2,813}=15.5$, $P=0.0001$), and also there was a significant interaction between generation number and lineage groups ($F_{4,813}=10.1$, $P=0.0001$). Post hoc analyses for each generation indicated that in the tutor generation and generation 1 all three lineages were significantly different from each other. In generation 2 there was no significant difference across wild-type and isolate lineages or between wild-type and heterospecific lineages, but there was a significant difference between isolate and heterospecific lineages (Figure 9).

We also tested how well our discriminant functions categorized syllables to the appropriate lineage. By the first generation the functions accurately assigned most of wild-type

syllables to the wild-type lineage, however only 34.5% of the isolate and 19.1% of the heterospecific syllables were categorized in the wild-type lineage (Table 3). By the second generation almost half (47.0%) of the isolate syllables were assigned to the wild-type lineage and 38.7% of the heterospecific syllables were assigned to the wild-type lineage. Collectively, with the above results, these data indicate that syllable features require at least one generation in the isolate lineage to resemble the wild-type syllables and at least two generations to resemble the wild-type syllables.

DISCUSSION

Throughout an experimental study of the cultural evolution of song, we explored the interaction between experience-independent biases in song learning and the nature of the tutor song within different lineages of zebra finches. We proposed that cultural evolution towards species-typical song features should be faster if the song of the founding generation has more species-typical features. In order to investigate this hypothesis, we developed three groups of lineages founded by song tutors singing wild-type zebra finch song (control group), isolate song, or song learned from a heterospecific (Bengalese finch).

We expected that, over generations, the isolate-tutored song lineages should shift toward wild-type song features faster than the heterospecific-tutored lineages, as the heterospecific song contains fewer species-typical song features. Our results partially met our predictions. We found that both experimental lineages, isolate-tutored and heterospecific-tutored, from the first generation shifted towards wild-type song features at the song-bout level. However, at the song-unit level the shift took one extra generation in the heterospecific-tutored lineage compared to the isolate-tutored lineage. At the syllable level, it took two generations for both groups to shift towards the wild-type zebra finch song features. Therefore, the rates of cultural evolution differ across lineages and levels of song analysis.

We were able to allocate the the zebra finches from the different linages and generations using a lineare discriminant function analysis. The discriminant functions determined the features that better differentiate the lineages, using the measurements obtained from the founder songs at the different song levels. Based on the specific features that characterized the different founder lineages, we developed three different models based on the level of song analysis. Using the founders of each group as at the initial model and then the next generations were added and

selected to one of the lineages. Specifically, we found that at the song-bout level, the features that differentiate the founder lineages were goodness of pitch (measure of pureness of a harmonic stack, entropy (noise) and song-unit density (song-units sang per second) (Table 2). At this level, the heterospecific founder group was characterized by having the lowest goodness of pitch, entropy and song-unit density. The Isolate founder group was characterized by highest goodness of pitch and entropy and the wild type founder group was characterized by having the higher song-unit density. At the song-unit level, the features that differentiate the founder lineages were related to the spectral power features, defined by mean frequency, peak frequency, amplitude, and frequency modulation (Table 2). Specifically, the Isolate founder group was characterized by high levels in all the spectral power features, followed by the wild type founder group and lastly, the heterospecific founder group. At the syllable level, frequency modulation and amplitude and goodness of pitch were the features that better differentiated the founder lineages. The heterospecific founder lineage was characterized by higher frequency modulation and lower amplitude and goodness of pitch, and frequency modulation lower than heterospecific founders, but similar to wild type.

Regardless of the founder lineage, most song features shifted quickly to wild-type within one or two generations. Even if the songs, after two generations, are still distinguishable by combinations of features, most of the individual feature parameters evolved quickly to completely overlap with wild type features. This suggests that zebra finches have a strong predisposition to imitate song elements with particular parameters, even though social interactions are important. However, using heterospecific stimuli reveals that the rate of cultural evolution to wild type depends on which songs the lineage starts with. Some features, e.g. bout length, seem to evolve more rapidly than others.

This study was based on a study developed by Feher et al. (2009). Even though we developed three different lineages and our levels of song analysis were different, we found similar results, with regard to the rapid cultural evolution towards a wild-type song in few generations, with most of the changes over the first generation. Also, features such as frequency modulation, amplitude modulation and goodness of pitch that differentiated the isolate lineage compared to the wild-type were also characteristic in the differentiation of our wild type founder group and heterospecific founder group.

401 Although the features we measured, and that were determined to discriminate the tutor
402 generation, evolved to wild type in two generations it is likely that songs from these lineages
403 would still be discriminable based on combinations of features. To determine this would require
404 behavioural testing to determine how zebra finches perceive and categorize the songs from
405 different lineages. Even though the songs evolved quickly based on linear discriminant function
406 analysis, they still sounded different to human ears. Perhaps with more generations the songs
407 would be totally indistinguishable from wild type. Or it may be that some species-typical
408 features would never emerge. Only future research would reveal this.
409

410

411 REFERENCES

- 412 Baptista, L. F., & Morton, M. L., (1981). Interspecific song acquisition by a white-crowned
413 sparrow. *The Auk: Ornithological Advances*, Volume 98, Issue 2.
- 414 Baptista, L. F., & Petrinovich, L. (1984). Social interaction, sensitive phases and the song
415 template hypothesis in the white-crowned sparrow. *Animal Behaviour*, 32(1), 172-181.
- 416 Baptista, L. F., & Petrinovich, L. (1986). Song development in the white-crowned sparrow:
417 Social factors and sex differences. *Animal Behaviour*, 34(5), 1359-1371.
- 418 Beecher, M. D. (2017). Birdsong learning as a social process. *Animal Behaviour*, 124, 233–246.
- 419 Beecher, M.D., & Brenowitz, E. (2005). Functional aspects of song learning in songbirds. *Trends*
420 *in Ecology & Evolution*, 20(3), 143–149.
- 421 Bolhuis, J. J., Okanoya, K., & Scharff, C. (2010). Twitter evolution: converging mechanisms in
422 birdsong and human speech. *Nature Reviews Neuroscience*, 11(11):747-59.
- 423 Brainard, M. S., Doupe, A. J., (2002). What songbirds teach us about learning. *Nature*,
424 417(6886):351-8.
- 425 Carouso-Peck, S., & Goldstein, M. H. (2019). Female Social Feedback Reveals Non-Imitative
426 Mechanisms of Vocal Learning in Zebra Finches. *Current Biology*, 29(4), 631–636.
- 427 Chaiken, M. L., Gentner, T. Q., (1997). Effects of social interaction on the development of
428 startling song and the perception of these effects by conspecifics. *Journal of Comparative*
429 *Psychology*, 111(4):379-92.
- 430 Chaiken, M., Condor, J. B., 2007. Song learning after isolation in the open-ended learner the
431 European starling: dissociation of imitation and syntactic development. *The Condor:*
432 *Ornithological Applications*, 109(4): 968–976.
- 433 Clayton, N. (1987). Song tutor choice in zebra finches and Bengalese finches: the relative
434 importance of visual and vocal cues. *Behaviour*, 104(3):281-299.
- 435 Clayton, N. S. (1989). The effects of cross-fostering on selective song learning in estrildid
436 finches. *Behaviour*, 163–175.
- 437 Dalziell, A. H., Welbergen, J. A., Iqic, B., & Magrath, R. D. (2014). Avian vocal mimicry: a
438 unified conceptual framework. *Biological Reviews*, 90(2), 643–668.
- 439 Doupe, A. J., & Kuhl, P. K. (1999). Birdsong and human speech: Common Themes and
440 Mechanisms. *Annual Review of Neuroscience*, 22(1), 567–631.
- 441 Fehér, O., Wang, H., Saar, S., Mitra, P. P., & Tchernichovski, O. (2009). De novo establishment
442 of wild-type song culture in the zebra finch. *Nature*, 459(7246), 564–568.
- 443 Funabiki, Y., & Konishi, M. (2003). Long memory in song learning by zebra finches. *The*
444 *Journal of Neuroscience*, 23(17), 6928–6935.
- 445 Holveck, M. J., Vieira de Castro, A. C., Lachlan, R. F., Cate, ten, C., & Riebel, K. (2008).
446 Accuracy of song syntax learning and singing consistency signal early condition in zebra
447 finches. *Behavioral Ecology*, 19(6), 1267–1281.
- 448 Houx, B. B., & ten Cate, C. (1998). Do contingencies with tutor behaviour influence song
449 learning in zebra finches? *Behaviour*, 135, 599–614.
- 450 Immelmann, K. 1972. The influence of early experience upon the development of social
451 behaviour in estrildine finches. *Proc. XV Int. Ornith. Congr., The Hague, 1970: 316- 338.*
- 452 Jarvis, E. D. (2007). Neural systems for vocal learning in birds and humans: a synopsis. *Journal*
453 *of Ornithology*, 148(1), 35–44.

- King, A. P. & West, M. J. 1983: Epigenesis of cowbird song – a joint endeavour of males and females. *Nature*, 305, 704–705.
- Konishi, M. (1964). Effects of Deafening on Song Development in Two Species of Juncos. *The Condor: Ornithological Applications*, 66(2), 85–102.
- Konishi, M. (1965). The Role of Auditory Feedback in the Control of Vocalization in the White-Crowned Sparrow. *Ethology*, 22(7):770 – 783.
- Lachlan, R. F., & Feldman, M. W. (2003). Evolution of cultural communication systems: the coevolution of cultural signals and genes encoding learning preferences. *Journal of Evolutionary Biology*, 16(6), 1084–1095.
- Lachlan, R. F., & Slater, P. J. B. (1999). The maintenance of vocal learning by gene-culture interaction: the cultural trap hypothesis. *Proceedings of the Royal Society of London B: Biological Sciences*, 266(1420), 701–706.
- Lachlan, R. F., Janik, V. M., & Slater, P. J. B. (2004). The evolution of conformity-enforcing behaviour in cultural communication systems. *Animal Behaviour*, 68(3), 561–570.
- Ljubičić, I., Bruno, J. H., Tchernichovski, O., (2016) Social influences on song learning. *Behavioral Sciences*, 7, 101-107.
- MacDougall-Shackleton, S. A., & Spencer, K. A. (2012). Developmental stress and birdsong: current evidence and future directions. *Journal of Ornithology*, 153(S1), 105–117.
- Marler, P., & Peters, S. (1977). Selective vocal learning in a sparrow. *Science* 198(4316):519-21
- Marler, P. & Zeigler, H. (2008). Neuroscience of birdsong. Cambridge University Press, 2008.
- Peters, S., Searcy, W. A., & Nowicki, S. (2014). Developmental stress, song-learning, and cognition. *Integrative and Comparative*, 54(4), 555–567.
- Poirier, C., Henry, L., Mathelier, M., Lumineau, S., Cousillas, H., & Hausberger, M. (2004). Direct social contacts override auditory information in the song-learning process in starlings (*Sturnus vulgaris*). *Journal of Comparative Psychology*, 118(2), 179–193.
- Price, P. H. (1979). Developmental determinants of structure in zebra finch song. *Journal of Comparative and Physiological Psychology*, 93(2), 260-277.
- Roper, A., & Zann, R. (2006). The Onset of Song Learning and Song Tutor Selection in Fledgling Zebra Finches. *Ethology*, 112(5), 458–470.
- Soma, M. F. (2011). Social factors in song learning: a review of Estrildid finch research. *Ornithological Science*, 10(2), 89–100.
- Spencer, K. A., & MacDougall-Shackleton, S. A. (2011). Indicators of development as sexually selected traits: the developmental stress hypothesis in context. *Behavioral Ecology*, 22(1), 1–9.
- Tchernichovski, O., Nottebohm, F., Ho, C. E., Pesaran, B., & Mitra, P. P. (2000). A procedure for an automated measurement of song similarity. *Animal Behaviour*, 59(6), 1167–1176.
- Thorpe, W. H. (1954). The Process of Song-Learning in the Chaffinch as Studied by Means of the Sound Spectrograph. *Nature*, 173(4402), 465–469.
- West, M. J. & King, A. P. 1988: Female visual displays affect the development of male song in the cowbird. *Nature*, 334, 244–246.
- Whiten, A., Ayala, F. J., Feldman, M. W., & Laland, K. N. (2017). The extension of biology through culture. *Proceedings of the National Academy of Sciences of the United States of America*, 114(30), 7775–7781.
- Williams, H., Kilander, K., & Sotanski, M. L. (1993). Untutored song, reproductive success and song learning. *Animal Behaviour*, 45(4):695-705.

501 **Acknowledgments**

502 This research was funded by a Discovery Grant from the Natural Sciences and Engineering
503 Research Council of Canada, and was conducted in a facility created with funding from the
504 Canada Foundation for Innovation. We thank Alice Cui, Michela Rebuli, Pralle Kriengwatana,
505 Tara Farrell, and Haruka Wada for assistance with this project.

506

507

508

509

510

TABLE 1: Descriptions of song features measured for the tutor and pupil crystalized songs.

	Song-Bout Feature	Feature Description
Performance	Song-Bout Duration	Length of the song-bout from the start to the end in milliseconds, including silence gaps between song-units.
	Song-Bout duration in Seconds	Length of the song-bout from the start to the end in seconds, including silence gaps between song-units.
	Song-units rate	Number of song-units per song-bout
	Song-Unit density per second	Number of song-units produced per second (number of song units multiply per 1 and divided by the song-bout duration).
	Song-Unit distance average	Mean obtained from the measure of the distance between the gaps from the end and the start of the following song-unit per each song-bout.
	Song-Unit Feature	Feature Description
Performance	Song-Unit Duration	Length of the song-unit from the start to the end in milliseconds.
	Song-Unit duration in Seconds	Length of the song-unit from the start to the end in seconds.
	Average syllable rate	Mean of the number of syllables per song-unit.
	Average syllable duration	Mean of the length of syllables in milliseconds.
	Rate of syllables per second (mean)	Number of syllables produced per second (number of syllables multiply per 1 and divided by the song-motif duration).
	Syllable Distance (mean)	Mean obtained from the measure of the distance between the gaps from the end and the start of the following syllable per each song-unit.
	Syllable	Feature Description
Performance	Syllable Duration	Length of each of the syllables. (Syllable: is defined as a continuous sound, bound by silence intervals).
	Syllable Distance	Mean obtained from the measure of the distance between the gaps from the end and the start of the following syllable per each song-unit.
Song-Bout, Song-Unit, Syllable Features		Feature Description
Spectral Features (Mean, Min, max, Standard deviation) spectral derivatives represent the change of power. Sound analysis pro manual.	Amplitude	It is the relative intensity of the sound, estimated at the frequency domain in unit decibels dB
	Mean frequency	It is the measure of the center of the distribution of power across frequencies.
	Peak frequency	It refers to the frequency of maximum power
	Goodness of pitch	It is an estimate of harmonic pitch periodicity. It indicates the periodicity of the sound. High goodness of pitch detects harmonic stack.
	Wiener entropy	It is a measure of the width and uniformity of the power spectrum.
	Frequency modulation FM	It is a measure based on time and frequency derivatives across frequencies.
	Amplitude modulation AM	It is the overall time-derivative power across all frequencies within a range. It indicates changes in the amplitude envelope of sounds
	Spectral continuity over time	It is the average duration over all contours. For each time window, the contours are detected, and the duration of each contour is measured.
	Spectral continuity over frequency	It is the average frequency range over all contours. For each time window, the contours are detected, and the frequency range of each contour is measured.

TABLE 2. Discriminant function parameters that maximally separated the three tutor lineages, for each of three levels of song organization (song bout, song unit, and syllable). Variables listed are those features that the discriminant function stepwise forward procedure extracted to maximally separate the three tutor song groups.

Standardized canonical discriminant function coefficients	Song-Bout	
	Function 1	Function 2
Song-Unit density per second	1.072	0.452
Goodness of pitch	1.666	-0.381
Minima Amplitude Modulation	0.991	0.779
Maxima Entropy	0.174	1.257

Standardized canonical discriminant function coefficients	Song-Unit	
	Function 1	Function 2
Peak frequency	1.22	-0.76
Frequency Modulation	0.167	1.157
Standard deviation Amplitude	0.96	0.39
Standard deviation mean Frequency	-2.06	-0.362

Standardized canonical discriminant function coefficients	Syllables	
	Function 1	Function 2
Goodness of pitch	0.525	0.515
Frequency Modulation	1.102	-0.687
Minima Peak frequency	0.237	0.383
Minima Spectral continuity over time	0.226	-0.437
Maxima Amplitude	1.091	-0.863
Amplitude	-0.545	1.253
Minima Frequency Modulation	-0.446	0.474
Maxima Goodness of Pitch	0.286	-0.663
Standard deviation mean Frequency	-0.698	0.164
Standard deviation Amplitude Modulation	-0.83	0.511

TABLE 3. Tests of how well the discriminant functions categorized songs from each generation to the the three different lineage groups.

Bout Discriminant Analysis

	Wild-type conspecific lineage			Isolate conspecific lineage			Heterospecific lineage		
	Wild-type conspecific	Isolate conspecific	Heterospecific	Wild-type conspecific	Isolate conspecific	Heterospecific	Wild-type conspecific	Isolate conspecific	Heterospecific
Tutor	6	1	0	1	5	0	0	0	6
Generation 1	8	0	0	2	4	2	5	1	7
Generation 2	3	2	1	2	4	2	5	5	4
Generation 3	3	0	0	1	1	1	4	0	3
Generation 4	0	0	2	0	1	1	1	0	3

Motif Discriminant Analysis

	Wild-type conspecific lineage			Isolate conspecific lineage			Heterospecific lineage		
	Wild-type conspecific	Isolate conspecific	Heterospecific	Wild-type conspecific	Isolate conspecific	Heterospecific	Wild-type conspecific	Isolate conspecific	Heterospecific
Tutor	5	2	0	1	6	0	0	0	6
Generation 1	5	2	1	1	7	0	1	3	9
Generation 2	2	4	0	3	3	2	0	11	3
Generation 3	1	1	1	1	0	3	1	4	3
Generation 4	0	2	0	0	1	1	3	0	0

Syllable Discriminant Analysis

	Wild-type conspecific lineage			Isolate conspecific lineage			Heterospecific lineage		
	Wild-type conspecific	Isolate conspecific	Heterospecific	Wild-type conspecific	Isolate conspecific	Heterospecific	Wild-type conspecific	Isolate conspecific	Heterospecific
Tutor	67	18	4	19	46	14	1	5	109
Generation 1	32	7	9	28	30	23	34	23	121
Generation 2	22	10	9	32	17	19	50	33	46
Generation 3	7	3	3	12	5	14	13	14	11
Generation 4	4	4	2	5	3	9	13	4	0

FIGURE LEGENDS

Figure 1. General timeline and experimental procedure. Young male zebra finches were isolated from their father, then assigned to one of three tutoring lineages (conspecific, heterospecific, or isolate song). For each generation, pupils from the prior generation were used as tutors for the next, to create three lineages over which cultural evolution through song learning could occur.

Figure 2. Example sound spectrograms of wild-type, isolate, and heterospecific songs from the initial tutor generation. Song units and song bouts are indicated by horizontal lines, and individual song syllables are indicated by lower case letters.

Figure 3. Example sound spectrograms from the founding tutor generation and the 4 offspring generations for the (A) heterospecific song lineage, (B) isolate song lineage, and (C) wild-type conspecific song lineage.

Figure 4. Plot of discriminant function values for song bouts from the tutor generation and first three generations of offspring in three lineages. Each point indicates the function values for the song bouts of an individual bird, with lineage indicated by symbol. Capital letters indicate the centroids of the tutor generation groups. In the tutor generation the three lineages show little overlap, but over generations the function values quickly overlap.

Figure 5. Distance from the centroid of the wild-type tutor group of song bouts from the tutor generation and first two offspring generations. Box plots indicate median and quartile range, and whiskers indicate range. Within each generation groups that share the same letter are not significantly different from each other. In the tutor generation the song bouts of all three groups differed from each other, but by the first generation there was no longer a significant difference across groups.

Figure 6. Plot of discriminant function values for song units (motifs) from the tutor generation and first three generations of offspring in three lineages. Each point indicates the function values for the song units of an individual bird, with lineage indicated by symbol. Capital letters indicate the centroids of the tutor generation groups. In the tutor generation the three lineages show little overlap, but over generations the function values quickly overlap. In generation 1, the isolate group song units overlap with the wild-type songs, but heterospecific songs do not overlap fully until generation 2.

Figure 7. Distance from the centroid of the wild-type tutor group of song units (motifs) from the tutor generation and first two offspring generations. Box plots indicate median and quartile range, and whiskers indicate range. Within each generation groups that share the same letter are not significantly different from each other. In the tutor generation the song motifs of all three groups differed from each other. In the first generation the isolate songs did not differ from wild-type, but the heterospecific songs did.

Figure 8. Plot of discriminant function values for song syllables from the tutor generation and first three generations of offspring in three lineages. Each point indicates the function values for the song syllables of an individual bird, with lineage indicated by symbol. Capital letters indicate

the centroids of the tutor generation groups. In the tutor generation the three lineages show little overlap, but over generations the function values quickly overlap.

Figure 9. Distance from the centroid of the wild-type tutor group of song syllables from the tutor generation and first two offspring generations. Box plots indicate median and quartile range, and whiskers indicate range. Within each generation groups that share the same letter are not significantly different from each other. In the tutor generation the song bouts of all three groups differed from each other, but by the second generation there was no longer a significant difference across groups.

Bengalese Finch

Isolate Zebra Finch

Wild Type Zebra Finch

Heterospecific Song lineage

Isolate song lineage

Wild-type Conspecific Song lineage

- ▲ Heterospecific
- Isolate
- Wild Type
- H Centroids heterospecific
- I Centroids Isolate
- W Centroids Wild Type

- ▲ Heterospecific
- Isolate
- Wild Type
- H Centroids heterospecific
- I Centroids Isolate
- W Centroids Wild Type

- ▲ Heterospecific
- Isolate
- Wild Type
- H Centroids heterospecific
- I Centroids Isolate
- W Centroids Wild Type