Chapter 2. Imitation of ISO song

Background & Rationale

Songbirds are masters of vocal imitation, capable of copying adult song with a nearly perfect accuracy. However, recent studies showed that despite the capability to imitate accurately, birds sometimes deviate from their model song. Examples include the social inhibition of imitation in zebra finches (Tchernichovski 1998) discussed in Chapter 1. Another revealing experiment in canaries took advantage of the fact that canary song is organized into phrases (repetitions of identical syllables) that make up the song. This syntax structure is found even in isolates. Gardner et al. (2005) trained young canaries with synthetic songs that consisted of syllable shapes comparable to normal canary song but organized in a "random walk" fashion. So rather than identical syllables and abrupt phrase boundaries, the syllables gradually turned into another type. The young birds learned these songs but as they matured, they switched to the species-typical phrase structure. Therefore, imitation and innate constraints are separate processes and can be segregated in time. In young birds, imitation prevails, but later in life, the innate biases come to dominate.

White-crowned sparrows are able to assemble complete songs when tutored only with phrase pairs (Rose et al. 2004). Although there is a strong innate predisposition to start a song with a whistle, young birds can be trained to sing in reverse order (whistle coming last) if trained with phrase pairs ordered in such a way. However, when trained with overlapping phrase pairs in the natural, forward order (AB, BC, CD, DE), birds easily assembled the whole sequence together (ABCDE). Birds trained with single phrases did not produce complete forward-ordered songs, although in 8 out of 9 cases they started their songs with a whistle (A) followed by a B-type phrase, which probably shows the extent of their innate predispositions. This example shows that imitation is aided by innate predispositions but can override them in case of a conflict.

There are two opposing forces acting on song learners that can have profound effects on the evolution of song cultures. On the one hand, random inaccuracies (“song-mutations”) and innovation among the young learners are the source of diversity, providing the raw material for the formation of local dialects. They can also facilitate the formation of individually distinct songs (perhaps indicating individual identity) within the dialect. On the other hand, biases in imitation (as in Gardner et al. 2005) might mirror innate constraints that keep song dialects within the WT range, otherwise imitation errors and innovation would cause unbounded variation over many generations or large geographical distances, which does not seem to the case (Marler & Tamura 1962). These postulated imitation biases could either alter the imitation of song features in a certain direction, or make it more likely that young birds imitate certain parts or certain features of songs.

What happens if we provide a young bird with an ISO tutor? Will the young bird exhibit imitation biases by preferential imitation of WT-like syllables or WT-like song features? We already know that juvenile males accept ISO song as a valid song model, and they imitate them even in the presence of WT tutors (Williams 1993). Moreover, in a colony, sons of ISO fathers imitate more of their father’s songs than sons of WT birds do of their own father’s songs (Williams 1993). However, studies that investigated the imitation of ISO song used coarse analysis methods, such as counting syllables in the pupil’s song that appear to be similar to those in the tutor’s song, and small or moderate imitation biases might not be captured using such methods.

In this dissertation, we tried using fine-grained quantitative methods, like the one we developed in Chapter 1 for assessing distances between WT and ISO songs. In Chapter 1 we showed that WT and ISO songs form distinct clusters along a continuum at the three timescales of song structure we investigated: spectral features, duration of acoustic state and song rhythm. We now examine the imitation of ISO songs and attempt to answer the question whether pupils of isolates show biased imitation regardless of tutor song and individual identity, and if they do this by selective imitation of WT-like syllables or modifications to existing syllables.

Methods

Animals

We trained 13 juvenile birds by ISO tutors one-to-one. We randomly selected hatchlings from 40 breeding pairs and did not let them hear songs after day 7 post-hatch. At this time, they were separated from their fathers and moved to a separate nursery area with their mothers and siblings. On day 30, the mothers were returned to the fathers and the juvenile males were paired with one of 6 isolate tutors. These tutors were randomly selected from our 17 ISO birds (isolation procedures are described in Chapter 1). The ISO tutor and his pupil were kept together for 90 days in a sound isolated chamber. To evaluate the effect of individual tutors, four of the isolate tutors were used 2-4 times to train unrelated pupils. Tutors' ages ranged between 140-1571 days (median age at beginning of first tutoring = 316 days). Additional training was performed serially (one-to-one) after confirming that the isolate tutor song remained stable over the tutoring period based on visual inspection & feature distributions.

Recording and Analysis

Sound recording was continuous during training using Sound Analysis Pro. However, since there were two sources of songs in every chamber (the pupil and the tutor), to obtain single recordings from every bird for positive song identification, we placed the pupil in a separate sound chamber, subsequent to the maturation of every pupil (around day 120), and recorded his song. Further analysis was done on these single recordings.

Data analysis & statistics were done using Matlab 7.0. Calculations of spectral features, duration of acoustic state and similarity measurements were done by Sound Analysis Pro 2. Detailed statistical tests are included in Appendix III.

Results

2.1 Visual assessment of imitation

First, let us examine the outcome of ISO tutoring by visually inspecting the sonograms and comparing tutor songs to all of their pupils' songs.

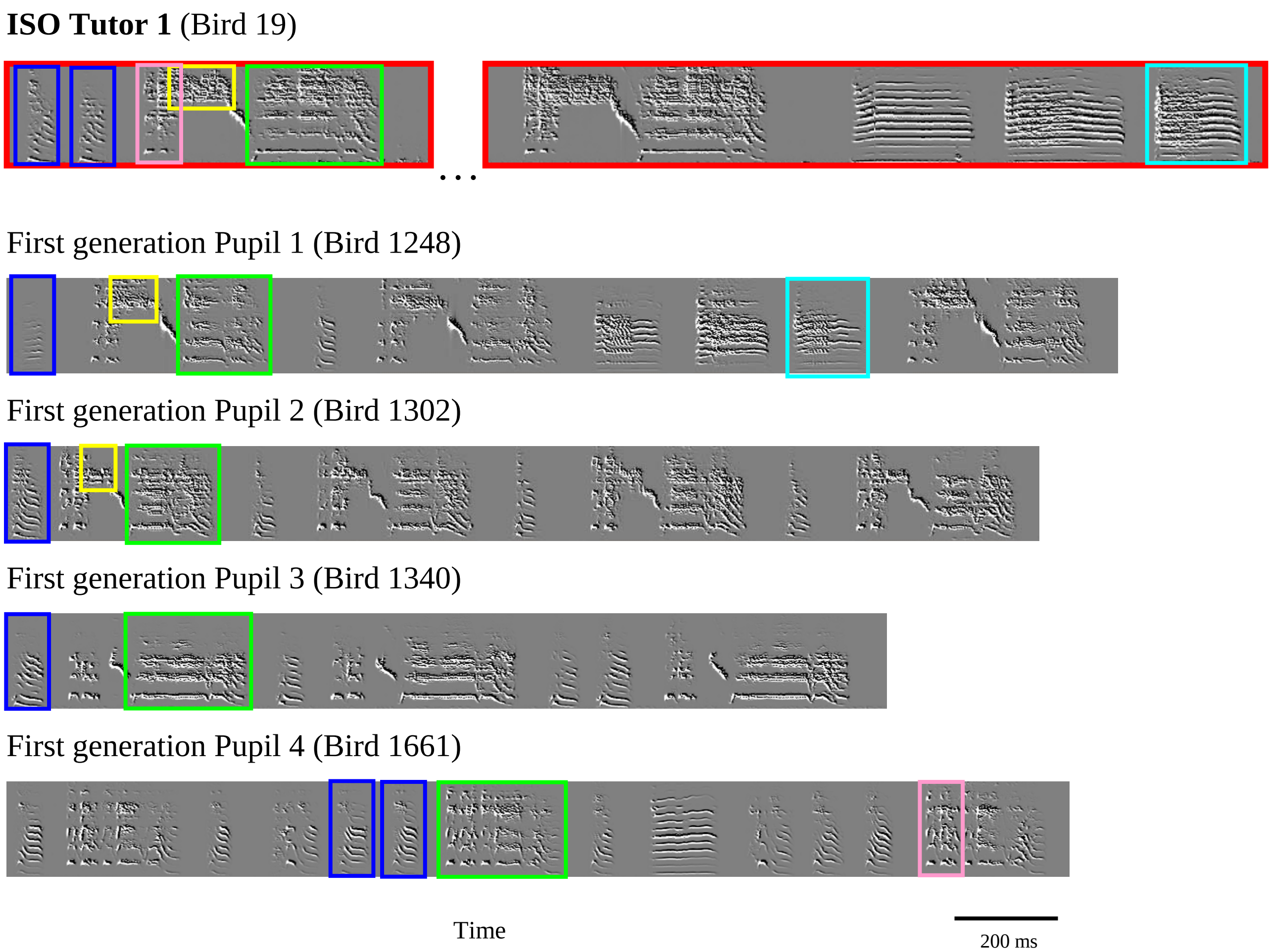


Figure 2.2 | Sonograms of Tutor 1 and his pupils. Song notes are marked with different colored rectangles (blue, pink, yellow and green in order in ISO song).

Tutor 1 (Fig. 2.2 upper panel) was our oldest tutor bird (age=1571 days at start of first tutoring) and his song was highly stable. He had a one-syllable motif and a rather stable bout structure. In addition to the simple bout structure, some of the notes are clearly ISO-like, with a narrowband noisy note (in yellow) followed by a short, high-pitched note and then a broadband noisy harmonic note (which sounds like a scratch). As seen above, all of the pupils of Tutor 1 imitated his song with more or less accuracy. Pupil 4 shows the least similarity. In his song, the only recognizable elements are the introductory syllable (blue rectangle) and the last, messy harmonic (green rectangle), but it seems like the pupil constructed this syllable from the spectral material of the first note of the complex syllable (pink rectangle). The duration of the yellow note, as well as that of the green one, is decreased in the songs of Pupil 1 & 2. Pupil 3 omitted the yellow note altogether. The syllables that dominate the song of the ISO tutor tend to be less abundant in his pupils – who added introductory notes and calls to the motifs. These songs and calls can all be found in the tutor's repertoire, but the tutor, in contrast to the pupils, sings them rarely.

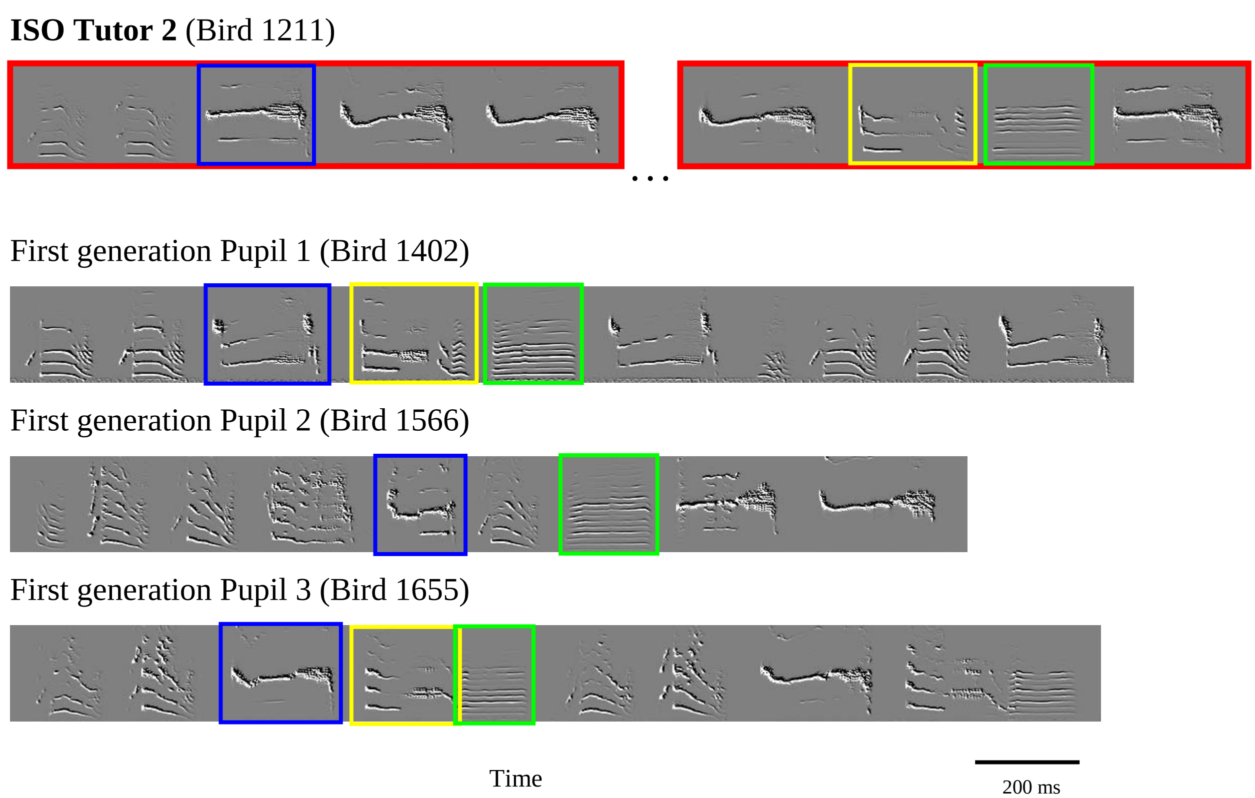


Figure 2.3 | Sonograms of Tutor 2 and his pupils. Important syllables are marked with different colored rectangles (blue, yellow and green in order).

Tutor 2's song (Fig. 2.3) contains one long (230 ms), high-pitched note (blue) that is very atypical of WT zebra finch song and two other syllables of similar duration that are harmonic-like (yellow and green). Pupils of Tutor 2 also imitated most of his song syllables. What is striking here is that none of the pupils produced repetitions of the blue syllable even though the tutor repeated this syllable at least 4 and up to 24 times in a song bout (median repetition rate=15). On the other hand, the syllables that were rare in the tutor's song (yellow and green squares) were much more frequent in all of the pupils' imitations. Pupil 2 shortened the blue syllable and Pupil 3 fused the yellow and green syllables.

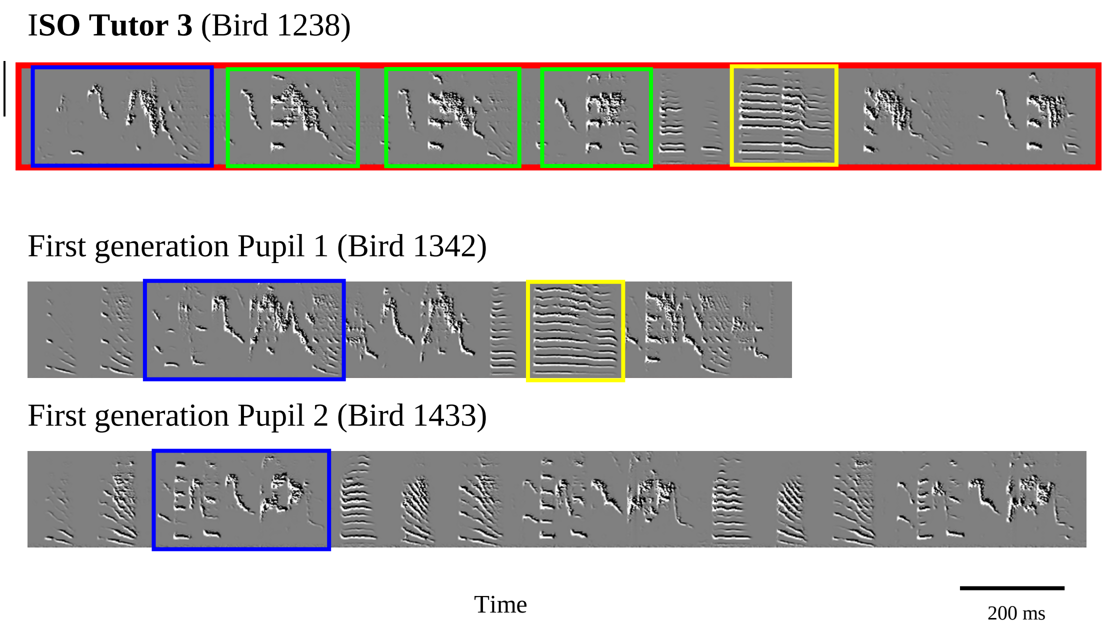
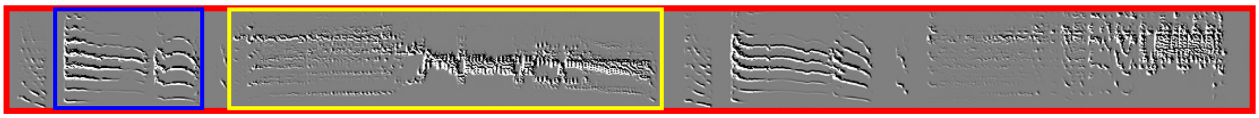


Figure 2.4 | Sonograms of Tutor 3 and his pupils. Important syllables are marked with different colored rectangles (blue, green and yellow in order).

Tutor 3 produced a song that is quite complex compared to other ISO songs (Fig. 2.4). For instance, it has fast transitions between different notes whose spectral shapes are quite complex. However, it does contain some features that are typical only to ISO song, such as the frequent back-to-back repetition of one syllable (green rectangles). Also, the harmonic sequences of syllables that appear occasionally in the bout are atypical e.g., the last harmonic syllable is long and includes a short stop in the middle, followed by a low amplitude ending (yellow). Both of these ISO-like features were changed by the pupils. We can observe reduction in Pupil 1 and omission in Pupil 2 of the long harmonic syllable, and a decrease in the number of the complex syllable repetitions to 2 Pupil 1 and 1 in Pupil 2.

ISO Tutor 4 (Bird 1247)



First generation Pupil 1 (Bird 1315)

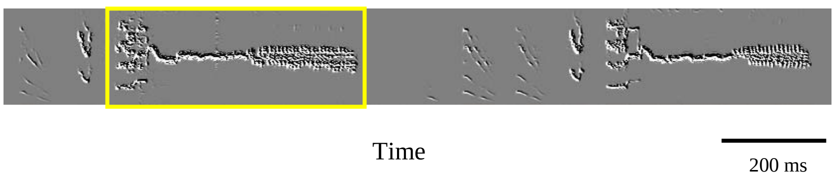


Figure 2.5 | Sonograms of Tutor 4 and his pupils. Important syllables are marked with different colored rectangles (blue and yellow in order).

Tutor 4 had the most abnormal song among our tutors (Fig 2.5). There is a harmonic element (blue square) that is long and call-like (although it was not included in every single bout) followed by an unstructured, broadband, scratchy syllable (in yellow) that is more than a second in duration, which is longer than the typical zebra finch song motif. This syllable is highly variable in the tutor in both duration and bandwidth and internal structure. The pupil of this tutor did not imitate the call-like harmonic, and greatly reduced the length and the bandwidth of the scratchy syllable. He has created a stable motif and bout structure out of a highly unstable song.

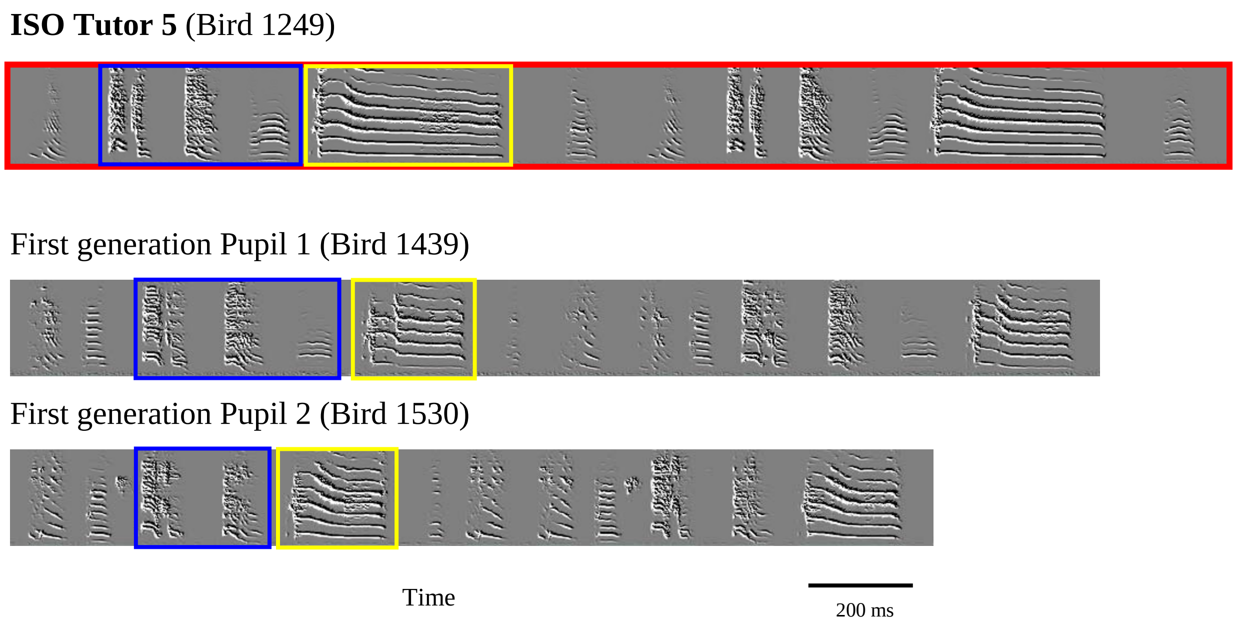


Figure 2.6 | Sonograms of Tutor 5 and his pupils. Important syllables are marked with different colored rectangles (blue and yellow in order).

Tutor 5 has a song (Fig. 2.6) with a few short syllables (blue rectangle) that are within the normal, WT range in duration as well as other spectral features, but the last syllable (in yellow) is an extremely long, call-like harmonic. Both pupils of this bird shortened the long call into a medium-length harmonic (a typical element of WT song) but copied all or most of the other syllables with high accuracy.

ISO Tutor 6 (Bird 1529)

First generation Pupil 1 (Bird 1622)

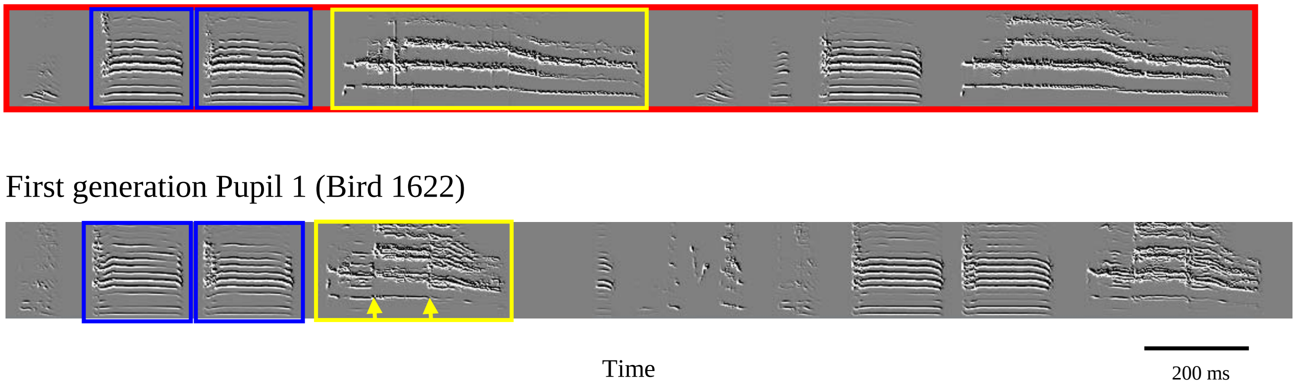


Figure 2.7 | Sonograms of Tutor 6 and his pupils. Important syllables are marked with different colored rectangles (blue and yellow in order).

Tutor 6 produced a very simple and highly abnormal, though quite stable, song. There are two long harmonics (blue) and an extremely long, high-pitched harmonic (yellow). The pupil imitated the harmonics accurately but shortened the long syllable. He not only shortened it, but also started to differentiate it into notes (boundaries marked with yellow arrows).

Summary of subjective inspection

Overall, there was a general tendency among pupils of ISO birds to shorten certain syllables that were of long duration. Repetitions of syllables disappeared in the songs of the pupils and some syllables became more complex or more stereotyped. In all cases, the pupils' motifs were highly stable: they always repeated the syllables in the same sequence with only minor variations, even in cases where their tutors sang extremely variable songs. Scratchy sounds were not imitated or greatly reduced in bandwidth and harmonic structure tended to be more stable in pupils than ISO tutors.

Before a formal test of whether these deviations from ISO tutor songs were robust and significant, we present a numerical summary of the imitation accuracy and syllable identities in tutor and pupil songs.

2.2 Imitation statistics

We used Sound Analysis Pro 2 to assess the accuracy of imitation. We segmented tutor and pupil songs into syllables and calculated imitation accuracy by using symmetric comparisons and then averaging across syllables. The determination of syllable types and their equivalents in pupils was done subjectively, but we confirmed our choices with accuracy measurements between all tutor and pupil syllables. Then we categorized syllables into complex or call-like syllables, once again subjectively, and calculated imitation rates for the different types of syllables. Table 2.1 contains the imitation statistics for each tutor and pupil pair.

Tutor	**19**	**19**	**19**	**19**	**1211**	**1211**
Pupil	**1248**	**1340**	**1302**	**1661**	**1402**	**1566**
Tutor: complex	1	1	1	1	3	3
Pupil: complex	1	1	1	1	3	3
% copied	100	100	100	50	100	100
% invented	0	0	0	0	0	0
Accuracy	81	63	72	39	88	76
Tutor: call-syll	0	0	0	0	0	0
Pupil: call-syll	1	1	1	3	2	3
Tutor: rare syll	2	2	2	2	2	2
#calls copied	3	?	?	?	2	2
#callsinvented	0	0	1	1	0	1

Tutor	**1211**	**1238**	**1238**	**1247**	**1249**	**1249**	**1529**
Pupil	**1655**	**1342**	**1433**	**1315**	**1439**	**1530**	**1622**
Tutor: complex	3	4	4	1	2	2	1
Pupil: complex	3	4	3	1	2	2	1
% copied	100	100	75	75	100	100	100
% invented	0	0	0	0	0	0	0
Accuracy	85.5	85.5	81	49	90	75	75.5
Tutor: call-syll	0	3	3	0	3	3	3
Pupil: call-syll	0	2	3	1	3	2	2
Tutor: rare syll	2	0	0	2	0	0	0
#calls copied	2	0	0	1	3	2	2
#calls invented	0	0	0	0	0	1	1

Table 2.1 | Imitation of isolate tutors in each tutor/pupil pair. Columns show the individual statistics for every tutor and his pupil. Per cent copied is the percentage of ISO tutor syllable types that were clearly copied by their pupil (judged subjectively), and where similarity measurements with SAP showed at least 50% significant similarity across the two syllables.

Table 2.1 shows that, with a couple of exceptions, imitation level was quite high, with moderate accuracy. Innovation was low and complex syllables were imitated in almost all cases. More birds invented call-like syllables than complex syllables.

Next, we examine these statistics across all tutor/pupil pairs. We calculated averages of imitation accuracy and imitations of complex and call-like syllables, and we summarized the results in Table 2.2.

	average	CV
Tutor: number of complex syllable types	1.85	0.58
Pupil: number of complex syllable types	1.92	0.54
% of complex syllables copied	92.31	0.17
% of complex syllables invented	5.08	2.44
Accuracy (mean across all syllables)	72.73	0.20
Tutor: number of short/long call types within motif	1.15	1.32
Pupil: number of short/long call types within motif	1.85	0.53
Tutor: # of rare syllables	1.23	0.82
# of calls + rare syllables copied	1.70	0.62
# of call types invented by pupil	0.38	1.32

Table 2.2 | Imitation of isolate tutors across all tutor/pupil pairs. Columns show the averages across all birds and the coefficient of variation. In the ISO tutors, "within motif" refers to syllables that appear in the middle of singing bouts (without an attempt to define motifs).

Table 2.2 shows that pupils imitated nearly all complex syllables (>92%) and their songs contained more complex syllables and less call-like syllables than the songs of their ISO tutors. Overall imitation accuracy was quite high (>72%), and there was little invention, but more complex than call-like syllables were invented.

2.3 Approximation of WT song features in pupils of isolates

In Chapter 1 we presented our results using PCA. We continue using PC1 and PC2 of song features at all three timescales to test whether pupils' imitations of ISO song significantly differ from our baseline of ISO songs (Fig. 2.8). PCA reveals that pupils are indeed intermediate between WT and ISO songs. The mean values of PC1 for the first generation pupils differed significantly from both ISO and WT means for the spectral-frame features and for DAS (p=0.018-0.001, n=13), but not for rhythm. Feature distributions of most individual pupil songs were closer to WT songs than were their tutor's songs (12/13 at at least one timescale, 10/13 at all timescales, FDR significance=0.01, binomial test, n=52, Appendix III).

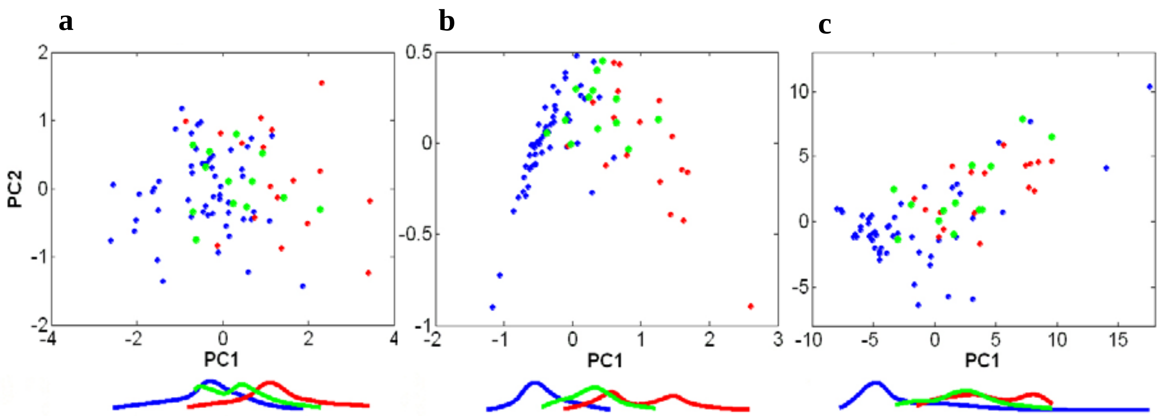


Figure 2.8 | Pupils imitating ISO song approximate WT distribution. PC1 and PC2 of combined spectral features (**a**), duration of acoustic state (**b**) and rhythm (**c**) in ISO birds (red dots, n=17), WT birds (blue dots, n=52) and pupils of isolates (green dots, n=13). Green dots fall between blue and red dots.

After confirming that imitation of isolate tutors is robust and significant, we now turn to examine the details and the possible biases in the imitations, using the methods developed in Chapter 1. We investigate the pupils' imitations at all three different timescales of song structure: spectral features, duration of acoustic state and song rhythm.

2.4 Spectral frame features in pupil songs

Spectral frame features describe the moment-to-moment changes in song. The three spectral features that provided the best separation between ISO and WT song were amplitude modulation (AM), frequency modulation (FM) and goodness of pitch. We will use these three features to investigate imitation of ISO song. Figure 2.9 presents the probability distribution histograms (top row) and the cumulative distribution histograms (bottom row) for all ISO birds (red lines), WT birds (blue lines) and pupils of isolates (green lines). These plots show that ISO sounds are lower in AM in general (Fig. 2.9a,b higher peak around zero in ISO), have more low-FM sounds (Fig. 2.9c,d high peak near zero in ISO) and lower goodness of pitch (high ISO peak near zero to the left of the WT peak Fig. 2.9e,f). In all panels, the green curves (pupils of isolates) appear to lie between red (ISO) and blue (WT) curves.

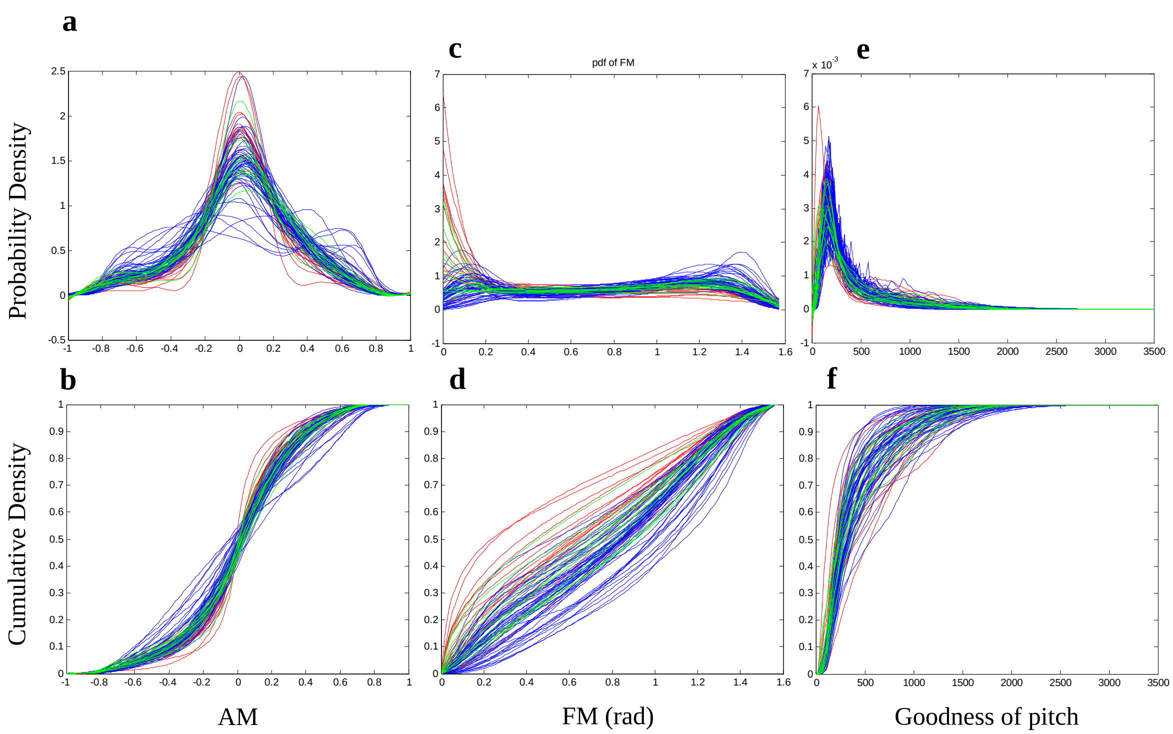


Figure 2.9 | Probability and cumulative distribution histograms of three spectral features in pupils of ISO tutors: AM (a,b), FM (c,d) and goodness of pitch (e,f). Red lines represent ISO birds (n=17), blue lines WT birds (n=52), green lines pupils of isolates (n=13). Probability distribution histograms in top row, cumulative histograms on bottom. (See Chapter 1 Methods for information on units of AM and goodness of pitch.)

In our further analysis, we use the scaled combination of the three features presented in Fig. 2.9. We used the PCA coefficients established for WT and ISO birds to project the song feature distributions of the first generation pupils on the PCA space of the WT/ISO songs (Fig. 2.10). The red dots represent isolate birds, the blue dots wild-type birds, and the green dots pupils of isolate tutors. The green dots appear intermediate between the red and the blue clusters, indicating a shift toward wild-type spectral frame features in pupils of isolate tutors. Histograms of PC1 distribution for each group (ISO, WT & pupils) are shown below the PCA plot, confirming that spectral feature distributions moved toward WT at all timescales. The mean values of PC1 for the first generation pupils differed significantly from both ISO and WT means for the spectral-frame features (p values 0.018-0.0126, respectively, n=13 pupils).

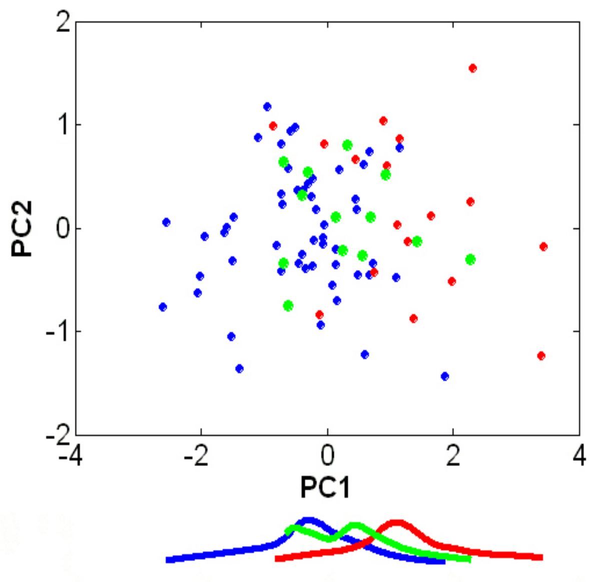


Figure 2.10 | PCA distribution of pupils of isolates is intermediate between ISO and WT distributions. PC1 and PC2 of spectral features of ISO (red, n=17), WT (blue, n=52) and first generation pupils of ISO birds (green, n=13). Histogram of PC1 values at the bottom. Shift towards WT features is indicated by the green dots positioned between the red and the blue dots.

Now we have seen that, overall, the spectral features of pupils are closer to WT spectral features than those of their ISO tutors. However, there has been no information so far about the effect of individual tutors. To assess whether there was progression towards WT in each case of tutoring, we drew arrows from each ISO tutor to all of his pupils (Fig. 2.11). Pupils of the same tutor are labeled by the same color. The location of the WT cluster in the PCA space is indicated by purple shading (shading represents the center of WT distribution based on density estimates; faint dots represent individual WT birds as in Fig. 2.10). As shown, most arrows point in the direction of the WT cluster. We did not observe any idiosyncratic effects of individual tutors on the size or direction of arrows, although pupils of ISO birds who were farther from the WT distribution made a longer shift in general (blue arrows) and variability across pupils (judged by the angle between arrows) was moderate. The only pupil that did not show a clear shift towards WT spectral features was the pupil of Tutor 4 (green arrow). This pupil imitated an extremely abnormal song (Fig. 2.5), and although his copy of the tutor syllable was shorter and of reduced bandwidth, the high-pitched extended note he produced is still quite abnormal. In some cases, like in the pupils of Tutor 2 (blue arrows), the shift towards WT-like features was very large. This was the tutor who sang the same high-pitched whistle-like syllable over and over in his song (Fig. 2.3), but his pupils reduced the pitch and emphasized his more WT-like syllables (yellow and green rectangles in Fig. 2.3) in their songs.

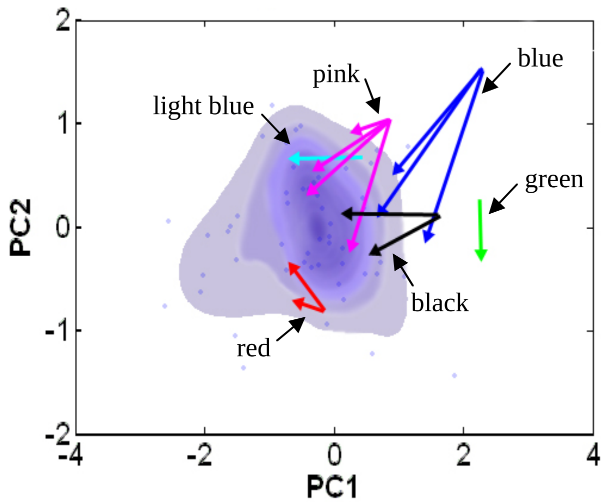


Figure 2.11 | Pupils of isolates shift towards the WT distribution. Different arrows indicate different tutor/pupil pairs, different colors different tutors. Blue shading shows the density estimates of the center of WT distribution.

We have seen that pupils showed a tendency to omit or alter ISO notes of certain spectral shape – e.g., “buzz” notes (ISO Tutors 1 & 4). Indeed, spectral features as judged by the first PC of the feature distribution were not copied accurately. Linear regression analysis of pupil versus tutor values in the first generation yielded a nonzero intercept and a slope slightly less than one (Fig. 2.12). The equality line, corresponding to faithful copying (pupil=tutor, dashed blue line), was rejected in favor of the alternative hypothesis represented by the linear fit shown in red ($P<0.001$, likelihood ratio test, $n=13$). Although copying is not accurate, it is consistent across tutors and pupils (as evidenced by the liner fit), so we can conclude that the imitation errors reveal universal biases.

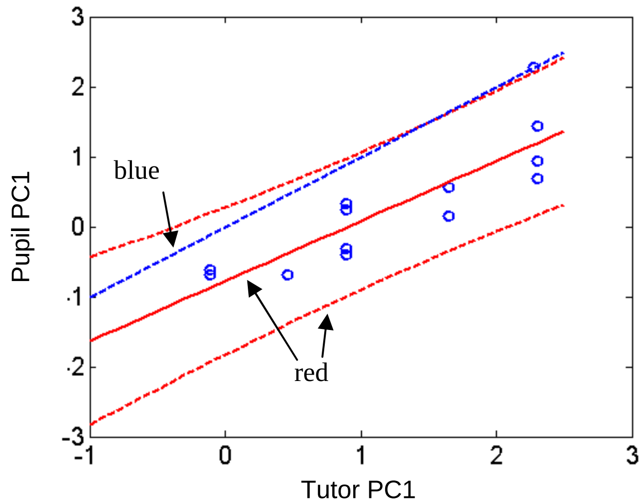


Figure 2.12 | Biased copying of spectral features. Correlation between first PCs of pupil versus tutor, indicating biased imitation. Dashed red line represents 95% confidence band, and the dashed blue line is the identity line.

2.5 Syllable and note duration in pupils of ISO birds

In this section, we will assess imitation in pupils of ISO birds with respect to deviations in note and syllable length as compared with their tutors' songs. We will investigate this question three different ways: by measuring individual syllable durations in tutors' and pupils' songs and comparing them, by calculating durations of acoustic state and by comparing longest to shortest note durations in ISO tutors and their pupils.

A. Syllable duration

When inspecting the sonograms, we noticed a common trend among pupils of ISO tutors, which was the decrease in the durations of abnormally long syllables (Figs 2.2, 2.5, 2.6, 2.7). To test for such an effect quantitatively we first identified syllables in tutor and pupil songs (as in Table 2.1) and then for each imitated syllable, we compared the duration to that of the tutor syllable. As an example, we present Tutor 5 and one of his pupil in Figure 2.13. Here, the long ISO syllable (red bar, mean duration=367ms, s.d.=29ms) was copied by a pupil, but its duration in the pupil's song was about 30% shorter (mean=243ms, s.d.=7.6ms).

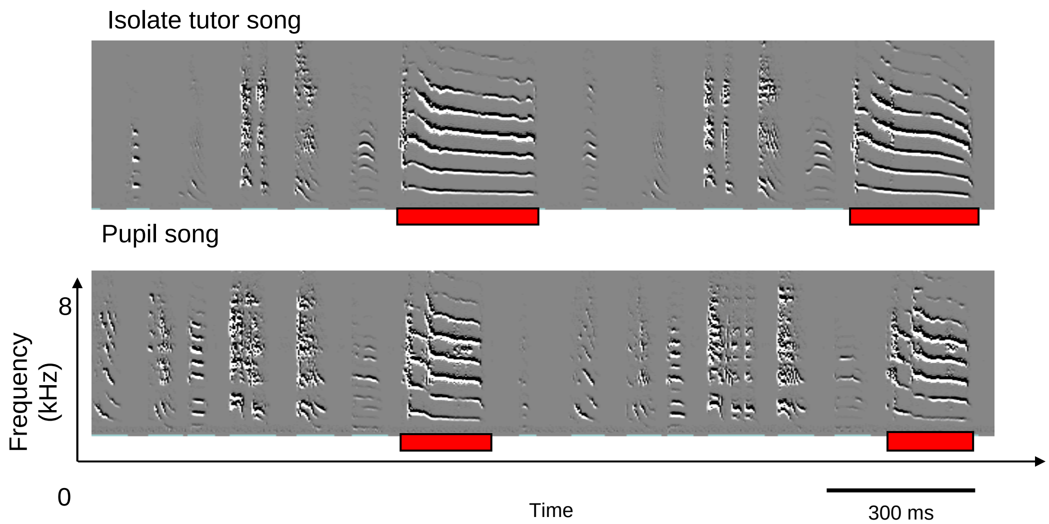


Figure 2.13 | Syllable shortening by pupil of isolate. The abnormally long harmonic syllable (underlined in red) is shortened by the pupil, even though the overall temporal frame (no decrease in motif length) and the durations of other syllables are preserved.

When we examined the relationship between tutor-pupil durations for all the syllables copied across birds, it became clear that not all tutored syllable copies are shorter, and we were interested in finding out whether it is true that the syllable decrease only applies to abnormally long syllables and not to all or any song syllables. Therefore, we plotted the syllable durations of ISO syllables against the durations of the respective imitations of these syllables (red dots in Fig. 2.14). Interestingly, the durations of pupil syllables accurately matched that of the corresponding ISO tutor syllables for syllable durations less than 230ms (r^2=0.98, slope=0.97, n=20 syllables). Copies of longer ISO syllables, however, were invariably shorter than the originals (r^2=0.84, slope=0.56, n=11 syllables). Overall, the range where durations of ISO syllables were accurately copied is similar to the range of WT syllable durations (25-75 percentile range = 67-180ms, n=52 WT birds). It is worth noting that across the entire range of ISO syllable durations, tutor-pupil durations were linearly related on a logarithmic scale (r^2=0.95, slope=0.84, n=31 syllables).

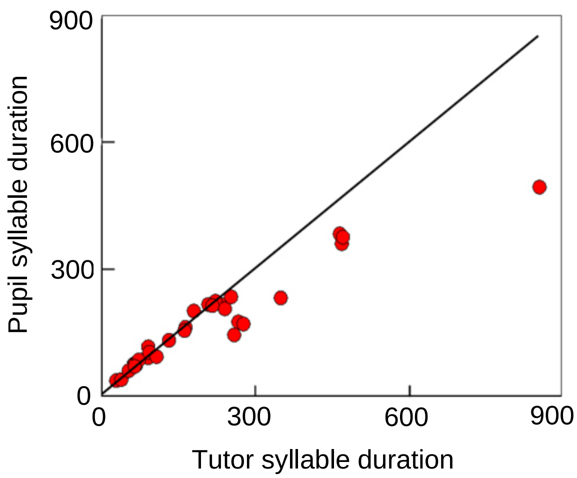


Figure 2.14 | ISO tutor syllable duration vs. duration of same syllable in pupil's song. Pupils faithfully copy syllable durations as long as they are less than about 230 ms. Longer syllables become shorter in pupils' songs.

B. Duration of acoustic state

To judge more generally if ISO songs change their acoustic states more slowly compared to WT birds, we developed the durations of acoustic state (DAS) measure (Chapter 1). We now examine if songs of pupil of ISO tutors are more similar to WT songs in DAS. Figure 2.15 presents cumulative distribution histograms ISO and WT birds and pupils of isolates. The green lines in Fig. 2.15b, which represent the pupils of isolates, fall in between the red lines (ISO birds) and the blue lines (WT birds). Therefore, we can conclude that there is a shift towards WT at the level of the note, too.

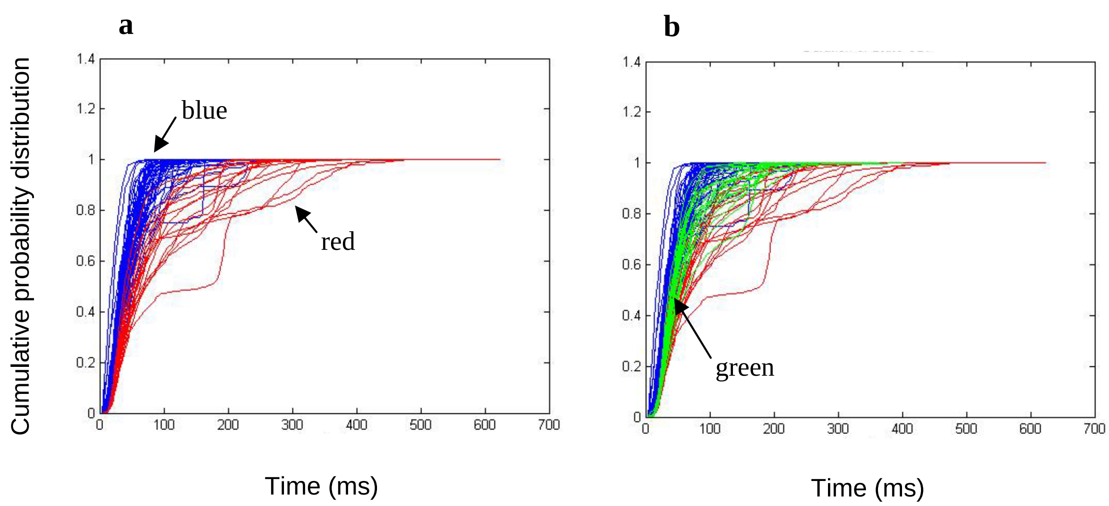


Figure 2.15 | Cumulative probability distributions of acoustic state durations in ISO birds, WT birds and pupils of isolates. a. Each line represents an individual ISO (red, n=17) or WT (blue, n=52) bird. **b.** Pupils of isolates are added in green (n=13).

Once again, we performed PCA to provide a better visualization (Fig 2.16) and statistical analysis. Results confirmed a significant shift towards WT in DAS values of pupils of isolates. The mean values of the first PC for the first generation pupils differed significantly from both ISO and WT means (p=0.0013 and p<0.0001, respectively, n=13 pupils). In addition, by connecting individual tutors and their pupils in this space (Fig. 2.16b), we can see that a shift toward WT occurred in every single case.

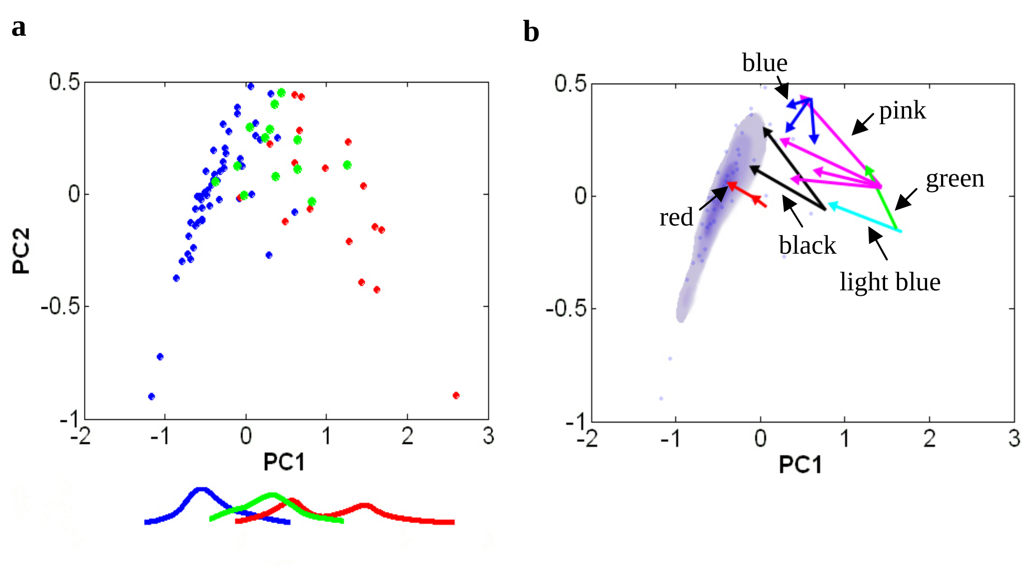


Figure 2.16 | PCA of DAS in ISO birds, WT birds and pupils of isolates. a. Each dot represents an individual ISO (red) or WT (blue) bird or pupil (green) bird. **b.** Arrows connect ISO tutors to pupils. Each arrow is a tutor/pupil pair, each color represents a different tutor.

C. Note duration ratios

Since this is the first time DAS is used as a descriptive tool, we investigated note-level changes in imitation using another feature, the distribution of note lengths. We calculated the ratio between the longest and shortest syllable within a bout. We found that this duration ratio was significantly higher in ISO tutors compared to their pupils (p<0.01 n=13, Wilcoxon sign test, Fig. 2.17).

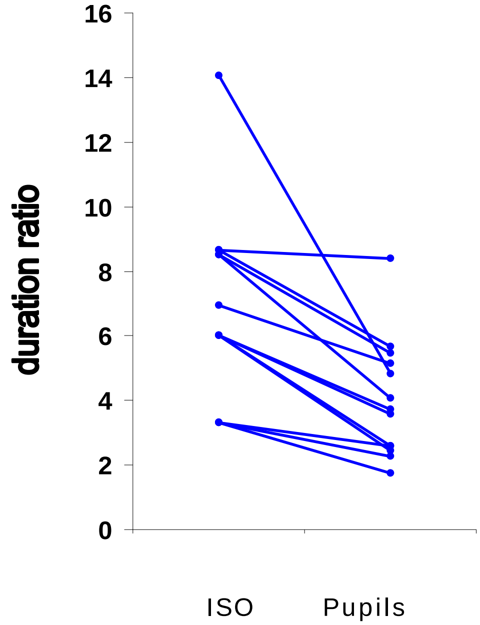


Figure 2.17 | Note duration ratios decrease in pupils of isolate tutors (n=13 ISO and pupil pairs). The left column shows the duration ratios of isolate tutors, the right column those of their pupils. In every case, the ratio decreased, showing that pupils had less variability in syllable duration.

In fact, note duration ratios of pupils of isolates fell right between those of WT and ISO birds. This is shown in Figure 2.18.

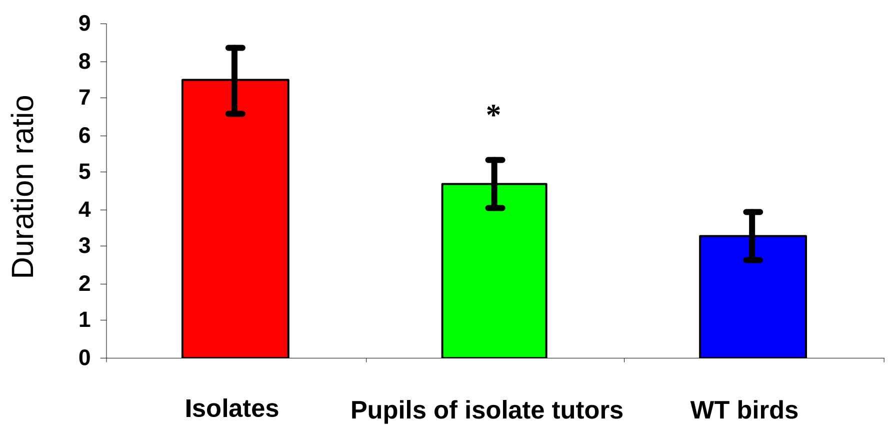


Figure 2.18 | Note duration ratios of pupils are intermediate to ISO and WT birds. Isolates (red, n=17) have the highest ratios, WT birds (blue, n=11) the lowest, and pupils of isolates are intermediate (green, n=10). Error bars indicate standard error. Asterisk above pupils indicates significant difference from ISO ($p<0.01$)

2.6 Bout-level features in pupils of ISO birds

In this section, we will examine the imitation of ISO song at the level of the bout. These include pupils' imitations of syllable abundance and song rhythm.

A. Syllable abundance

A feature that pupils consistently changed was the abundance of dominant syllables. ISO songs often contain back-to-back repetitions of the same syllable type, sometimes dominating the song in abundance (e.g., Tutor 2, Fig. 2.3). In contrast, syllable stuttering is rare in WT song. In WT songs, syllables are nearly always organized in a stable sequential order, repeating only once in a song motif. We now examine this global reorganization of syllabic structure by taking a closer look at Tutor 2 and his first pupil (Fig. 2.19). Tutor 2 sang back-to-back renditions of two syllable types (denoted as *A* and *B*). In the tutor, the abundance (relative frequency) of syllable *B* was 81%. The pupil imitated both syllables but syllable B was altered (B') and its relative frequency decreased to 19%.

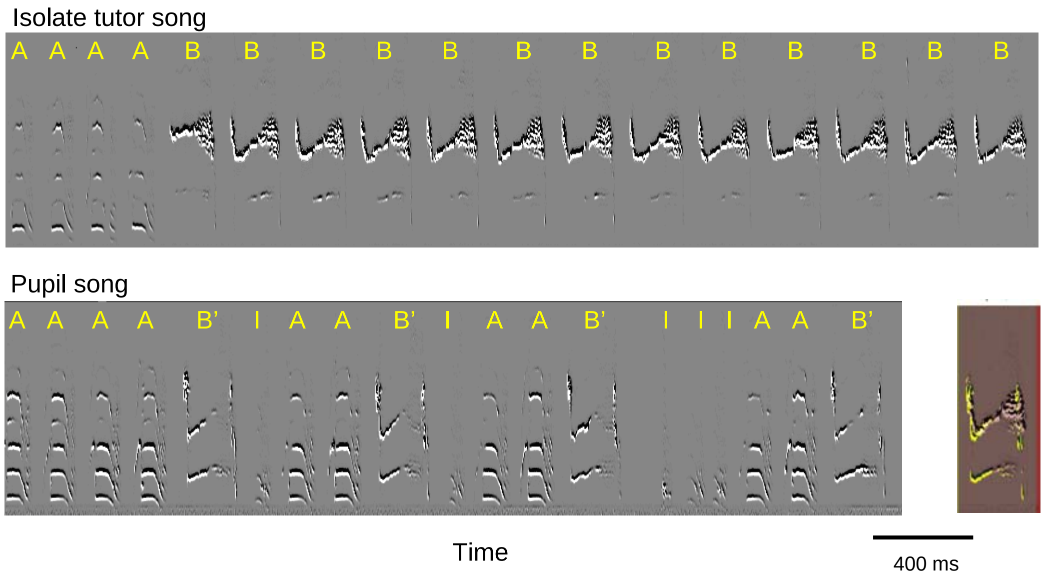


Figure 2.19 | Syntactic reorganization in pupil of isolate. Tutor 2's song consists of two syllables, both are repeated consecutively (AAAABBBBBBBBB…), but his pupil did not copy the repetitions of syllable B, rather, he constructed a motif with a sequential organization of alternating syllables (AABAAB).

Across birds we found that ISO tutors varied markedly in the relative frequency of their most abundant syllable (mean=41%, range=10-84%). These "most abundant syllables" were copied by all 13 pupils, but the relative frequencies of the same syllables in pupils' songs were significantly lower and less variable (mean=20%, range=12-30%, $p<0.01$, Wilcoxon sign test). Interestingly, when the relative frequency of the most abundant tutor syllable was 30% or lower, the relative frequency of the same syllable in the pupil song followed the tutors' values (Fig. 2.20**,** $r^2=0.77$, slope=0.85, p=0.02, n=6 birds). However, for syllables in the tutor song with relative frequencies higher than 30%, there was no correlation ($r^2=-0.02$, slope=0.04, NS, n=7 birds), and relative frequencies in pupil song decreased to 20-30%. Overall, the ranges of relative frequencies in pupil songs corresponded to that of WT songs, where they rarely reach 30%.

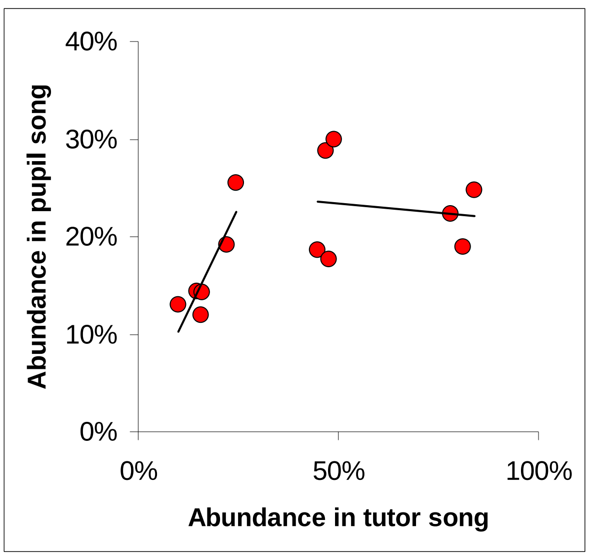


Figure 2.20 | Abundance of dominant syllable in ISO tutors' and their pupils' songs. Red dots indicate individual syllables. Syllable abundance was imitated faithfully in the normal WT range (up to 30%), but syllables dominating ISO songs too much were reduced in relative frequency to the WT level.

This shift in abundance may also indicate the formation of motifs in pupils of isolates. Zebra finch song motifs contain 3-8 syllables on average, which, when repeated only once as in WT motifs, would correspond to a 13%-33% syllable abundance for each syllable. As pupils exhibit this range, it is possible that, as opposed to their ISO tutors, they sing stable motifs.

B. Song rhythm

Some of the previously described alterations in ISO features, such as the decrease in back-to-back syllable repetitions and the shortening of syllable duration inevitably lead to changes in the global temporal structure of the song. Pupils' songs sound shorter, more structured and rhythmic. We will now attempt to quantify these rhythm changes using the technique introduced in Chapter 1.

We now turn directly to PCA, because rhythm spectra of birds singing different songs cannot be combined in histograms. In Chapter 1, we showed that ISO songs have less structure than WT songs and that when the first two principal components are plotted against each other, they occupy different regions of the distribution. We now plot the PC1 and PC2 values of pupils of ISO birds in the same graph (Fig. 2.21). As shown in the figure, most pupils (green dots) lay intermediate to the ISO (red) and WT (blue) birds. However, the separation between pupils and their ISO tutors did not reach statistical significance for rhythm.

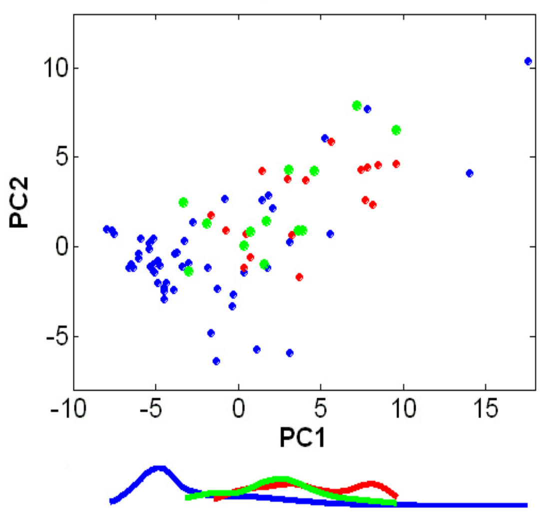


Figure 2.21 | Rhythm PCA distribution of pupils of isolates. PC1 and PC2 of rhythm of ISO (red, n=17), WT (blue, n=52) and first generation pupils of ISO birds (green, n=13). Histogram of PC1 values at the bottom. Shift towards WT features is indicated by the green dots positioned between the red and the blue dots.

Even though the effect was not significant in rhythm, it is useful to look at how the rhythm shifted between each individual tutor/pupil pair. Therefore, we present the same data with arrows drawn between tutors and pupils, as we had done for DAS and spectral features (Fig. 2.22). We can see that for some tutors (pink and black, representing Tutor 1 and Tutor 5, respectively) the rhythm shift is strong in the WT direction. Pupils of Tutor 2 and 3 (blue and red arrows, respectively) showed inconsistency in this measure, and the pupils of Tutor 4 & 6 (light blue and green arrows, respectively) shifted in the opposite direction. However, these results are very doubtful, because these last two tutors had extremely abnormal, long syllables and basically no motif at all, but their pupils sang much shorter and more stable songs (see Fig. 2.5 and 2.7). These temporal changes must have surely manifested themselves in a more structured song rhythm. In fact, to the ear, the pupils' songs sound much more normal than the tutors' songs. Consequently, the negative results are due to shortcomings and inconsistencies in our measure of song rhythm rather than a shift towards ISO rhythm.

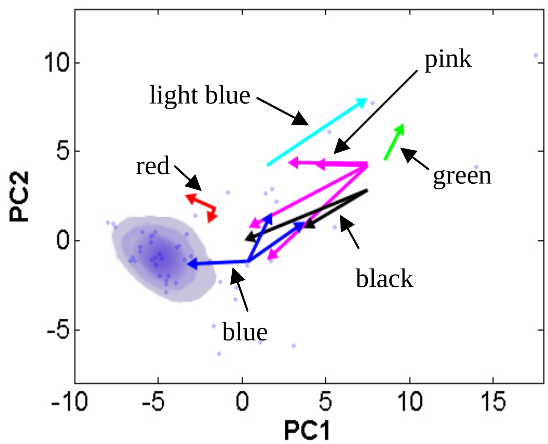


Figure 2.22 | Rhythm changes in PC1 and PC2 between ISO tutors and pupils. Arrows originate at tutors and point to pupils. Different color arrows represent different tutors. Shaded area is the density estimate of the center of WT distribution.

2.7 Conclusions

We have shown that pupils of ISO birds, while imitating their tutors, modify their songs in ways that are consistent across pupils' and tutors' songs, shifting towards WT song features. In other words, pupils learning their songs from isolates sing more normal, WT-like songs than their tutors. To statistically confirm this finding, we computed pair-wise Euclidean distances between the cumulative feature distributions of each pupil and his tutor, and between the pupil and our library of 52 WT birds. 12 of 13 pupils showed significant progression toward WT at at least one of the three timescales of song structure (FDR significance level=0.01, binomial test with n=52). Ten birds showed significance

(FDR level=0.01) at all timescales. Therefore, most birds did shift towards WT features regardless of what song feature is considered.

The implications of this shift in pupils of isolates are great, because it provides evidence to the existence of innate biases that shape song imitation and may form the basis of evolutionary changes, such as dialect formation and preservation. Perhaps most interestingly, these biases surfaced immediately upon providing a tutoring environment but not without it. Isolates do not show these biases when they develop their own songs, but their pupils, even though they are confined in some sense by the abnormal tutor song, are able to employ them to shape and modify their imitations of the tutor songs.

We find it remarkable that the syllables invented by the isolates, who still use auditory feedback to develop these syllables (Price 1979), are not subject to the biases that we see in their pupils. It appears as if the isolate bird has “no problem with” hearing himself singing a bizarre scratchy syllable, that pupils of isolates show no biases against imitating bizarre ISO syllables, and yet when the pupil copies these syllables, he shifts their features toward WT. We therefore interpreted the shift toward WT songs observed in the pupils as an outcome of *imitation biases*. Namely, biases that are associated with the imitation of songs – not with production per se, and not during the selection of song model (which would have resulted in selective imitation of some syllables over others).

Imitation biases are likely innate. We will refer to these biases as innate hereafter, although we cannot exclude the possibility that some involvement of early social experience with the mother affected the development of such biases.

It was surprising that we saw such a strong shift towards WT features in pupils of isolates. There are instances of rapid dialect changes in the wild, but they usually involve the addition of new song elements that become “fashionable” in a population. Such processes have been described in Puget Sound white-crowned sparrows (Chilton & Lein 1996) and humpback whales (Noad et al. 2000). What is very interesting in our results is that pupils did not add or delete sounds much, but rather, manipulated the existing cultural variant and fit it to an “innate idea of what it should be like” – which is the WT structure.

We will next explore what happens to these songs in a recursive tutoring paradigm, where the pupils of ISO tutors become tutors themselves and so on. We will track the evolution of songs over a few generations of song learners.