Freedom and Rules: The Acquisition and Reprogramming of a Bird's Learned Song

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Canary song is hierarchically structured: Short stereotyped syllables are repeated to form phrases, which in turn are arranged to form songs. This structure occurs even in the songs of young isolates, which suggests that innate rules govern canary song development. However, juveniles that had never heard normal song imitated abnormal synthetic songs with great accuracy, even when the tutor songs lacked phrasing. As the birds matured, imitated songs were reprogrammed to form typical canary phrasing. Thus, imitation and innate song constraints are separate processes that can be segregated in time: freedom in youth, rules in adulthood.

The songs of adult songbirds consist of stereotyped units, called syllables, acquired by imitation and arranged in sequences characteristic of each species. An understanding of how syllables are sequentially arranged to form songs is of general interest because the learning and reordering of stereotyped motor patterns is the basis of speech and may underlie other aspects of motor control (1, 2). The neural basis of vocal production in songbirds is beginning to be understood (3–6), but the mechanisms that program the sequential order of syllables remain unknown.

The typical song of the canary (*Serinus canaria*) consists of tonal whistles ("syllables") that differ in duration and frequency modulation. Each syllable is repeated many times, forming a "phrase" (Fig. 1). Phrases are produced in variable order. Young canaries reared in the presence of singing adults will closely imitate the song of the latter (7), a process that takes 6 to 8 months; in these birds, incipient phrasing is already present by 60 days of age (8). However, canary-like syllables and phrase structure also develop in the absence of imitation and even in the absence of hearing, although under these conditions the repertoire of syllables is uncommonly small (7, 9, 10). Normal phrased song can be rapidly induced in very young, isolated male canaries by exposing them to adult levels of testosterone. This rapid emergence of adult-like song is shown in Fig. 1.

We wondered whether young canaries would imitate songs that lacked phrasing and violated a key "rule" of canary song. To address this question, we synthesized two kinds of songs that never occur in the repertoire of an adult canary (11) and exposed young canaries to these songs while isolating them from exposure to natural canary song. We built the first abnormal tutor song by generating a 30-s "random walk" through a space of syllable shapes comparable to that of normal canary song (Fig. 2A). Each syllable in the song contained a modulated tone with harmonics, filtered to create a timbre similar to that normally produced by the birds themselves (12). However, the overall sequence was unlike the phrases in normal adult canary song, because each syllable in the sequence differed

slightly from the preceding and following one in shape and duration. The second synthetic song designed for this study was based on three repeats of a 5-s "glissando"; in a glissando, the same basic syllable occurs in descending frequency steps. These synthetic songs are illustrated in sonogram form and can be heard as sound files (11).

The canaries of the Belgian Waterslager strain that we used were born in soundproof chambers and were reared solely by their mothers, which at that time did not sing. At 25 days of age, males were transferred to individual soundproof chambers, where they heard a playback of a tutor song every 2 hours during the daylight photoperiod, which followed the natural outdoor cycle. Ten birds were exposed to the random walk, and six to the glissando; this exposure was continued for the duration of the experiment. Sound was recorded continuously and automated tools were used to detect and extract songs, discarding brief calls and other cage noises. Thus, we had for each bird a complete record of its vocal ontogeny.

In contrast to normal song ontogeny where phrase structure is apparent already in early plastic song (as in Fig. 1B), for these birds the earliest imitations contained long sequences of dissimilar sounds. In the random-walk group, six of 10 birds achieved remarkably accurate imitations of the first 10 s of the tutor song. These juvenile imitations, exemplified in Fig. 2B, demonstrate that juvenile canaries can accurately memorize and imitate atypical
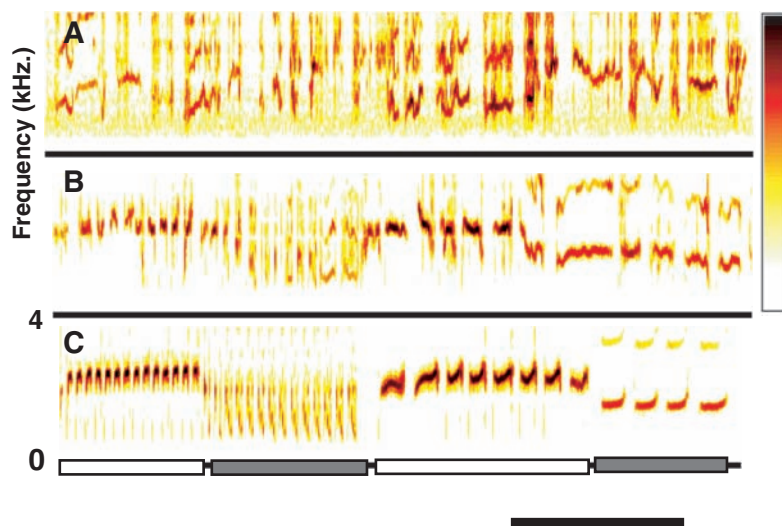
Fig. 1. A canary reared in social isolation develops normal syllable and phrase structure. The normal time course for song learning is 6 to 8 months, but an implant of testosterone at 2 months of age induced development of normal adult song structure over the course of a single week. The changes that took place during this week recapitulate song ontogeny in accelerated form and are illustrated with time-frequency sonograms (scale bar at bottom, 1 s). (A) The first stage, recorded at 75 days of age, is highly variable, with no evidence of stereotyped syllables and only the faintest suggestion of phrase structure. (B) At 80 days, most of the syllables that the bird will use as an adult already appear in a recognizable if still variable manner, organized in emerging phrases (13). (C) At 82 days, 1 week after testosterone implant, the variability of syllable structure is reduced, forming adult-like song. White and black rectangles indicate phrases consisting of repeats of stereotyped units, the syllables of adult song. Syllables are 20 to 200 ms in duration, and phrases are typically 1 s in length. The color scale presents the loudest sound in each panel as dark brown.

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songs that lack phrasing. This 10-s imitation represents a considerable achievement, because the summed duration of all unique sounds (not counting repetitions) produced in aviary-raised Belgian Waterslager canaries is on average 2.7 s (8). The result of this tutoring experiment indicates that song imitation in juvenile canaries can be remarkably unconstrained. However, song development did not end there, because our training had delayed but not eliminated the use of phrases (see below).

Between 150 and 250 days of age (i.e., in late fall and early winter), six of the 10 birds tutored with the random-walk model achieved a clearly recognizable imitation; there was considerably less imitation in the other four, and we did not follow their song thereafter. In pilot work, we had noticed that after birds achieved a clear imitation of the random-walk model, the approach of the breeding season brought a gradual change toward the typically phrased song of aviary-reared juveniles. This change occurred more slowly or rapidly and with varying degrees of completeness in different individuals. Therefore, beginning in January, we exposed two of the six birds that imitated the random-walk model to a more abrupt change in photoperiod, and additionally gave them a testosterone implant to overcome any factors that might slow the transition to a full breeding condition. Recall that in isolated juveniles, the change from early plastic song to phrased adult song was induced by this kind of forced transition to adult breeding status (Fig. 1).

The two testosterone-treated birds displayed a marked rearrangement in song structure. The nature of this change is shown in Fig. 2, C and D. In the early imitated songs, the birds had retained the syllable order as it appeared in the tutor song (Fig. 2C). In the transition to adulthood, a subset of the imitated sounds was serially repeated to form phrases,

and in successive songs these phrases occurred in different orders (fig. S2), as in the song of aviary-reared birds. Because the transition from the imitated random-walk song to phrased song involved some of the original imitated syllables, we refer to this change as "reprogramming," a term that implies a reordering of existing material. For the bird whose imitated song is pictured in Fig. 2C, an automated survey (11) of all songs produced in the month of March yielded no match to the tutor sequence; the song with the best residual similarity to the tutor is illustrated in Fig. 2D. For this bird, the inferred innate rules forced a complete reprogramming of the imitated song. This reprogramming occurred in the absence of any exposure to normal canary song and in the presence of continued tutoring with the random-walk model.

In the four birds exposed to natural lengthening of days and spared the testosterone implant, the transition to phrased song occurred more gradually. All birds that achieved detailed copies of the tutor song in the juvenile period reprogrammed their songs as they approached sexual maturity.

Each bird's record of song development included 5000 to 30,000 songs, and therefore it was necessary to develop an automated methodology for comparing each song to the tutor (11). This method recognized something we have all experienced: A given word can be spoken faster or slower or at higher or lower pitches, and the same is true for canary song. The similarity score was based on the well-defined pitch contours of syllables (13), with the use of dynamic time warping to find the best match between two sounds (14, 15). The alignment algorithm (Fig. 3A) shows how the extent of similarity with the tutor model changed over time in the six birds that imitated the random-walk model. This similarity started very low, increased as the tutor model

was mastered, and then declined as the birds approached breeding condition. In parallel with the late decline in match to the tutor, there was an increase in phrased repetition (11), quantified in Fig. 3B.

In addition to the emergence of syllable repetition, the transition to adulthood involves a change in the distribution of syllable shapes that are most commonly used in song. This change includes reduction of variability in some syllables as well as attrition of other syllables, resulting in songs built from a small subset of stereotyped syllable forms. This change is shown in Fig. 3, C to F, by density plots of syllable mean pitch and duration. At the peak of imitation, the distributions indicate a broad range of syllable characteristics. One month later, after exposure to breeding conditions, the distributions indicate a transition toward a more categorical syllable distribution comparable to that of aviary-raised adult canaries (fig. S4).

The transition from juvenile imitation to adult song included, for all birds, a period when both the imitated song and phrased song coexisted. Most of the six birds that imitated the random-walk model maintained some capacity to produce the juvenile imitations in adulthood. Intact fragments of up to 6 s of the juvenile imitation were occasionally integrated into adult songs consisting of phrased repetitions (fig. S1). When a bird produced both song types in adulthood, some of the same imitated syllables could be found in both song types. We infer that the order of these syllables was determined sometimes by imitation and at other times by an innate phrase-forming program. Moreover, the switch between these two states of serial ordering occurred over short periods of time and even within the same song (fig. S2).

The capacity to produce both song types throughout adulthood was more pronounced in

Fig. 2. Canaries achieve detailed imitations of an unnatural "random walk" song but reprogram these imitations in the transition to adulthood. (**A**) Synthetic tutor song. (**B**) Example of the best imitation achieved by 200 days of age. (**C**) Imitation produced by another bird at 250 days. (**D**) An automated survey (11) revealed that by 300 days, the bird whose song is pictured in (C) produces only phrased song. In the phrased song, a subset of the imitated syllables [small rectangles in (C)] are produced in phrased repetitions [stretched rectangles in (D)]. Scale bars at lower right: time, 1 s; frequency, 3 kHz.
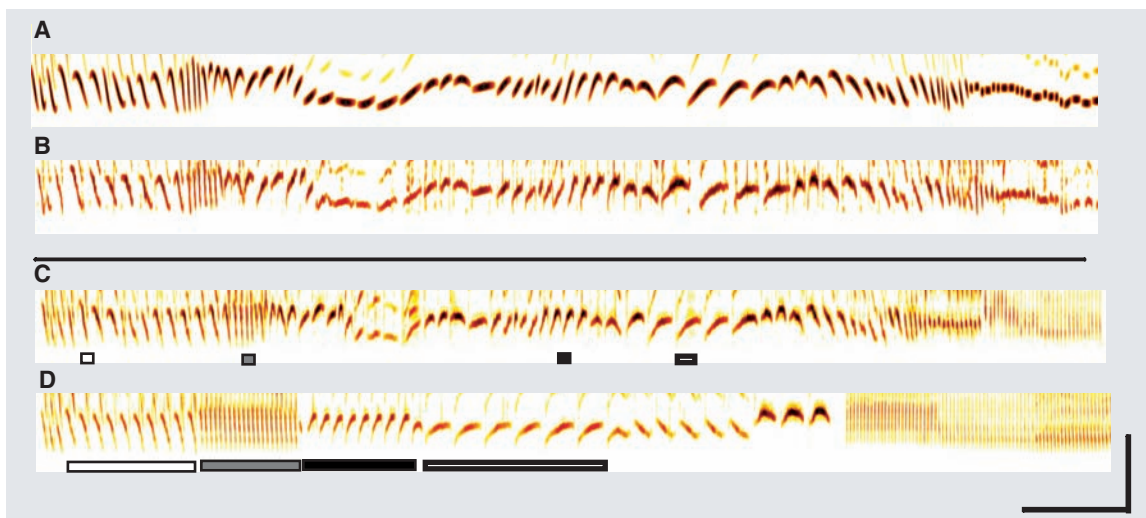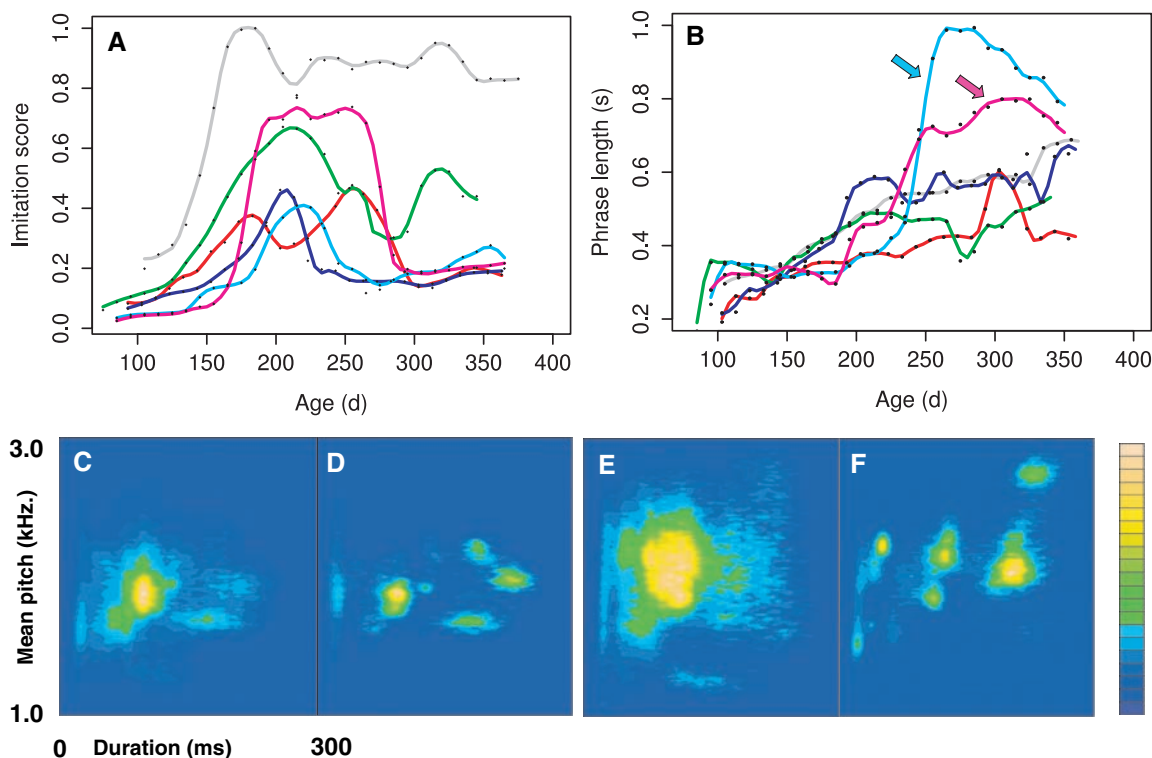
the group of birds exposed to the glissando song (Fig. 4). In this group, accurate imitations achieved in the juvenile period were preserved throughout adulthood (fig. S3), even after exposure to exogenous testosterone. In adulthood, glissando imitations were produced side by side with phrased songs; these phrased songs consisted of syllables apparently drawn from the glissando song as well as of im-

provised syllables that the bird had not heard before. As for the first group, the switch from imitated sequence to phrased song and back could occur within a single song (Fig. 4, B and C) (fig. S3), which suggests that imitated syllables could be accessed by two different programs.

We have established the following: (i) Juvenile canaries can accurately perceive,

memorize, and imitate long, atypical sequences of unique sounds arranged in a single linear order. (ii) As these birds approach sexual maturity, they produce a subset of these sounds as serial repetitions in the phrased manner typical of adults. (iii) Adults that produce both unphrased and phrased song can switch from one to the other over short time scales. Such quick reprogramming is reminiscent of the

Fig. 3. (**A**) Time course of model imitation using the alignment algorithm (*11*). Every pupil's song is scored on the length and acoustic match of the best fragment that can be aligned to the tutor song. For each week, the scores of the best imitations, defined as the 90th percentile by rank (i.e., the typical best imitation, not the very best that the bird can do), are plotted after smoothing with a running average over 30 days. This plot is essentially unchanged when plotting percentiles in the range 90 to 100%. Each color represents one of six birds. (**B**) Average phrase length increases over time (*11*). The two birds with the sharpest increase in phrase length (light blue and magenta traces, arrows) re-

ceived exogenous testosterone treatment starting at 210 and 180 days, respectively. (**C** to **F**) Density plots (*11*) illustrate a transition in how the two testosterone-treated birds apportioned their singing time in terms of syllable characteristics. Each panel is calculated from the durations and mean pitches of 60,000 syllables selected from random times in the month of peak imitation [(C) and (E)] and in the subsequent month [(D) and (F)]. Colors are normalized separately for each panel, with yellow indicating the

highest concentration of singing time. At the peak of imitation, singing time is still distributed across a range of duration and pitch characteristics. In adulthood, this distribution becomes fragmented into distinct clusters; each cluster in the density plot is typically populated by multiple syllable types that have similar duration and mean pitch values. Patterns in (D) and (F) are qualitatively similar to those obtained from colony-reared canaries singing adult song (fig. S4).

Fig. 4. Canaries imitate an extremely unnatural "glissando song." In adulthood, the birds rapidly alternate between imitation of the glissando and songs that reprogram a subset of the glissando syllables to form phrases (phrase boundaries are indicated by tick marks.) (**A**) Tutor song. (**B**) Glissando imitation and phrased structure produced in the same song by an 8-month-old canary after 2 months of testosterone treatment. (**C**)

Example of glissando imitation and phrased structure produced in the same song by another adult, age 11 months, after 2 months of testosterone treatment. Sound files in (B) and (C) were adjusted with a running amplitude normalization to better illustrate loud and quiet syllables in the same color scale. Scale bars: time, 1 s; frequency, 3 kHz.
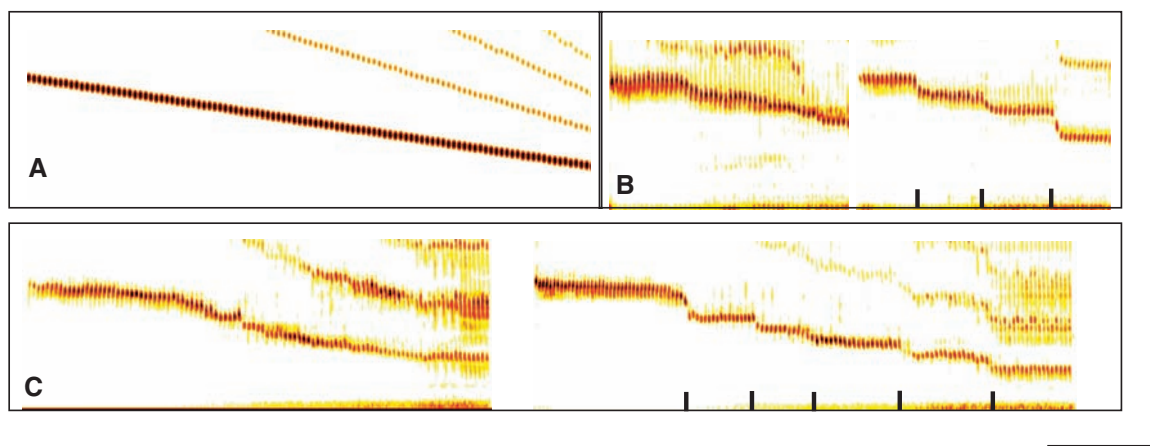
flexibility of phoneme rearrangement in human speech and is an aspect of vocal prowess in birds that had not been described before. Given the many parallels in the ways in which birds and humans go about vocal learning (*16–18*), there may also be common neural mechanisms underlying the ways in which they can access and sequence the learned vocal units.

Classical ethologists have remarked on the fact that, among songbirds that learn their song but are not usually thought of as mimics, model imitation is usually restricted to conspecific models. Imitations of other species, which occur rarely during the juvenile stage, are usually expunged by sexual maturity (*19–21*) and thus do not compromise a species' "song universals" (*22*). It has been known, too, that some songbirds master as juveniles more songs than they will sing as adults (*21, 23, 24*). These are examples of how selective attrition of juvenile song can lead to the final repertoire of adults.

Our work with canaries sheds light on this editing process. Normally, the rules of adult canary song guide vocal ontogeny, so that phrasing is already in evidence in early juvenile song, even in the absence of external models (Fig. 1). However, this mechanism for developmental guidance is separate from the ability to learn a song, and the two can be temporally uncoupled. During the first half of their juvenile life, our canaries were free to imitate sounds that violated the rules of nor-

mal canary song. As the breeding season approached, this material was reorganized both by attrition of some syllable variants and by imposing a new syntax, so that it now conformed with the rules of adult song. This order of occurrence—freedom to learn followed by rules of expression—may tell us something important. Vocal learning may have evolved not just by relaxing innate programs for vocal ontogeny and linking them to external models and auditory feedback, but also by delaying the onset of adult strictures, allowing for a period of vocal experimentation and play. A glimpse of this early evolutionary step may be found in young birds learning their song.

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