Are song sequencing rules learned by song sparrows?

Susan Peters [a] , Jill Soha [a] , William A. Searcy [b, *] , Stephen Nowicki [a]

[a] *Department of Biology, Duke University, Durham, NC, U.S.A.*
[b] *Department of Biology, University of Miami, Coral Gables, FL, U.S.A.*

ARTICLE INFO

Although the effects of learning on song structure have been extensively studied in songbirds, little attention has been given to the learning of syntax at the level of song sequences. Here we investigate song syntax learning in two cohorts of hand-reared song sparrows, *Melospiza melodia*: an isolate group, consisting of four males raised with no exposure to external song models, and a trained group, consisting of 17 males exposed to recorded song sequences during the sensitive period for song learning. The isolate males followed three syntactical rules previously described for field-recorded song sparrows: (1) they produced their song type repertoires with eventual variety, repeating a song type multiple times before switching to another; (2) they cycled through their repertoires using close to the minimum number of bouts; and (3) they showed consistent preferences for singing certain of their song types more than others. The trained males were tutored with sequences with exaggerated eventual variety and cycling patterns and no usage preferences, but their syntax was little affected by any of these training features. One syntactical pattern that was affected by external experience was the rule that long bouts of a song type are followed by long recurrence intervals before that type is produced again. Isolate males showed no bout length/recurrence interval correlations while trained males showed reduced correlations relative to field-recorded males, implicating learning in the development of the normal pattern. Other songbird species have been found to preferentially use song type transitions as adults that they were tutored with as juveniles, but the trained song sparrows in this study showed no evidence of such effects.
© 2022 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Song development in songbirds is a canonical example of vocal learning (Marler, 1970a; Thorpe, 1958), with many parallels to another canonical example, speech acquisition in humans (Doupe & Kuhl, 1999; Marler, 1970b; Soha & Peters, 2015). In studies of birdsong development, most attention has been given to vocal production learning, in which the acoustic structure of vocalizations is influenced by experience with the vocalizations of other individuals (Janik & Slater, 2000). Vocal production learning includes the learning of syntax at the level of the ordering of notes or syllables within songs (Lipkind et al., 2013; Todt & Hultsch, 1998). Learning of syntax at the level of the ordering of song types into sequences is considered usage learning rather than vocal production learning (Janik & Slater, 2000). Some species of songbirds in which individuals possess repertoires of multiple song types have been shown to follow syntactical rules at the song sequence level (Catchpole & Slater, 2008), but whether these rules are learned from external models is largely unknown. Here we address this question in the song sparrow, *Melospiza melodia*, a species whose adult syntax has been especially well studied (Nice, 1943; Nordby et al., 2002; Searcy et al., 2022).

One focus of previous studies of syntax development in songbirds has been the distinction between immediate and eventual variety. This distinction has to do with bout structure, where 'bout' refers to the consecutive renditions of a specific song type. In the immediate variety pattern, a singer switches song types after nearly every song, thus producing mainly single-song bouts (e.g. ABCDE …). In the contrasting eventual variety pattern, the singer usually repeats a song multiple times before switching to a new type, so that single-song bouts are uncommon and mean bout lengths are considerably greater than one (e.g. AAABBBBBCCCC …) (Catchpole & Slater, 2008; Hartshorne, 1956). The development of these patterns has been studied in nightingales, *Luscinia megarhynchos* (Todt & Hultsch, 1996, 1998), a species in which individual males possess large repertoires of up to 200 or more song types, which they normally sing with immediate variety. Hultsch (1991) trained a control group of young nightingales with recorded songs presented with immediate variety and an experimental group with songs presented in a mix of one-song, three-song and six-song bouts. As adults, both groups produced most of their songs with immediate variety, but the experimental group used an eventual variety pattern significantly more often than did the controls. These results suggest that the immediate variety rule in nightingales is partly learned and partly innate.

* Corresponding author.
  E-mail address: wsearcy@miami.edu (W. A. Searcy).

Another focus of previous studies has been the learning of transition preferences; that is, preferences for switching from one particular song type to a second specific type. In some songbirds, the first-order transitions used in adulthood are copied at least in part from those heard early in life. Hultsch and Todt (1992, 1996) tutored young hand-reared nightingales with recorded sequences of songs and found significant similarities between the ordering of song types in the tutor sequences and the ordering used by subjects in adulthood. Copying of song type order improved with increasing numbers of exposures to a training sequence, although even a single exposure had some effect. The first song sequence ever heard by the young nightingales had a particularly strong impact on adult syntax, a phenomenon dubbed the 'primer effect' (Hultsch & Todt, 1996). In similar experiments, two of two marsh wrens, *Cistothorus palustris*, and one of three chaffinches, *Fringilla coelebs*, disproportionately reproduced as adults the first-order transitions they were exposed to as juveniles (Kroodsma, 1979; Riebel & Slater, 1999). Thus, transition preferences are affected by learning in at least some songbirds.

Syntax at the level of song sequencing has been described more comprehensively in song sparrows than in most songbirds, making possible a more extensive exploration of the role of learning in syntax development in this species. Song sparrows have repertoires of 6–12 song types, which they sing following the eventual variety rule (Nice, 1943; Saunders, 1924). Song sparrows have long been suggested to cycle through their repertoires (Nice, 1943; Nordby et al., 2002), in the sense that an individual tends to produce a bout of each of its song types before returning to any type. Recently, Searcy et al. (2022) confirmed for a sample of 21 song sparrows recorded free-singing in the field that cycle lengths — the number of bouts used to produce the entire repertoire — were always lower than in random sequences and took the minimum possible value in a majority of cases (e.g. eight bouts for a repertoire of eight song types). Males did not cycle through their repertoires in a set order; instead, even adjacent cycles showed low similarity in the first-order transitions used and transition preferences were rarely consistent for individuals across days (Searcy et al., 2022).

Cycling without following a preferred order implies the existence of long-distance dependencies, in which choice of the next song type is influenced by the order of song types over the whole of the preceding cycle (Searcy et al., 2022). A pattern that demonstrates long-distance dependencies more directly is the 'bout length rule', which mandates that longer bouts of a song type are followed by a longer 'recurrence interval' before another bout of that type is given. Searcy et al. (2022) found that 20 of the 21 song sparrows in their sample showed positive correlations between bout lengths and subsequent recurrence intervals as predicted by this rule. The bout length rule is also supported by data showing that the longer the most recent bout of a particular song type, the less likely song sparrows are to match playback of that type (Searcy et al., 2019). A final aspect of song sparrow syntax is that males show usage preferences for certain of their song types that are consistent over days (Lapierre et al., 2011; Searcy et al., 2022). Usage preferences are driven more by variation in bout lengths than by variation in number of bouts, although both play a role (Searcy et al., 2022).

Here we investigate how learning affects the acquisition of syntax in song sparrows using two cohorts of hand-reared subjects. One cohort was reared with no exposure to song sparrow song. Isolate male song sparrows have previously been shown to develop songs that are abnormal in many aspects of acoustic structure (Kroodsma, 1977) but that nevertheless retain some species-typical features (Marler & Sherman, 1985). The syntax used in song sequences produced by such isolates has not previously been described. The second cohort of young males was exposed during the sensitive period for song learning (Marler & Peters, 1987) to recorded sequences of song that obeyed some species-typical syntactical rules but not others. The syntax exhibited in adulthood by these two cohorts of hand-reared birds can be compared to the syntax of wild-recorded birds of normal experience to see whether and how experience affects syntax development. Using this approach, we investigate the development of five syntactical patterns: eventual variety, cycling, the bout length rule, usage preferences and transition preferences.

METHODS

We compared the syntax of song sequences across three groups of song sparrows. The first group, consisting of hand-reared males that were isolated from any exposure to adult song sparrow song, we refer to as the 'isolate males'. The second group, consisting of hand-reared males that were trained with recorded sequences of song sparrow song, we refer to as the 'trained males'. The third group, the 'field-recorded males', consisted of 21 free-living males whose syntax was previously described by Searcy et al. (2022).

Isolate Males

The four birds in this group were collected during May 1996 from three nests in Durham County, North Carolina, U.S.A. The birds were collected 3–5 days posthatch and taken to a laboratory at Duke University. Starting at approximately 18 days posthatch, the birds were housed in individual sound-attenuation chambers (58 × 41 × 36 cm, Industrial Acoustics AC-1, New York, NY, U.S.A.). The birds were maintained in these individual chambers on naturally varying daylengths through May 1997, when their songs were recorded. Recordings were made with a Realistic 33-1070A omnidirectional microphone connected to a Marantz PMD 221 cassette recorder via a Yamaha MLA7 mic to line amplifier with a Digitech RDS 1900 digital delay to facilitate automatic recording. We analysed song sequences from two recording dates for each subject, all 1 week apart. We used for analysis samples taken after lights on. Because the number of isolate birds was small to begin with, we included all recording sessions from all the isolate birds in the analysis regardless of the sample size of the number of songs per session.

Trained Males

The 17 birds in this group were collected in May 2013 from eight nests in Crawford County, Pennsylvania, U.S.A. for a study of song learning and cognition (Anderson et al., 2017). Subjects were collected as nestlings at 3–6 days posthatch, before the start of the sensitive period for song learning in song sparrows (Marler & Peters, 1987). The birds were transported to a laboratory at Duke University in Durham, North Carolina, U.S.A., where they were housed in groups except on days when they were recorded. The males were initially housed with females from the same nests but were separated before they began producing subsong. Throughout, males could hear other males of the same cohort sing, which has been shown to affect song development in some songbird species (Leitner & Catchpole, 2007). Birds were maintained on seasonally varying daylength through the duration of the study. Starting at approximately 10 days after hatching and continuing for 12 weeks, the birds were tutored twice daily with recorded song sparrow songs. Training thus spanned the period in which the great majority of songs are memorized in hand-reared song sparrows (Marler & Peters, 1987). Training stimuli were presented at 75–80 dB (at 30 cm) from an Advent Powered Partner AV570 speaker that was positioned 0.6–2.0 m from the subjects.

All training stimuli incorporated a set of 32 song types previously recorded at the Crawford County sites where the subjects were collected. Each song type was presented once per training session in a bout of 24 repetitions over a 4 min period with 1 min of silence between successive bouts. Sequences in the training stimuli (e.g. 24D–24H–24F …) thus followed the eventual variety rule. A new order of song types was chosen randomly for each week of the 12 weeks of training; the chosen order was then maintained for all training sessions for all subjects for that week. Note that training stimuli followed the cycling rule, in that the 32 song types were presented in the minimum possible number of bouts in all 12 sequences that were used; however, cycle lengths were much longer than in natural sequences (which contain at most 12 distinct song types) and only a single cycle was presented per training session. The training stimuli followed the transition preference rule, as the same randomly chosen transitions were presented repeatedly over the course of a week, and only a minority of the possible transitions were used across the 12 weeks. Some specific transitions were included by chance in more than one training sequence. The training stimuli did not follow the bout length rule, in that there was no relationship between bout length for a song type and the length of the interval before that song type was presented again. The training stimuli also did not follow a usage preference rule, as all song types were presented in exactly equal numbers.

The birds in this sample, collected as nestlings in 2013, were recorded in 2014, 2015 and 2016. Recordings were made between the last week of April and the first week of June each year. For each subject in each year, we analysed two samples of songs, recorded on average 8 days apart (range 3–14 days). Recordings were made using a Shure SM57 cardioid microphone, an M-audio Profire 2626 audio interface, an Optiplex Dell Computer and Sound Analysis Pro software SAP2011. Recording started 1 h before lights on and lasted 3 h. To make these samples more comparable to those from the field-recorded males, which were all made during daylight hours, we included in the analysis only songs produced after lights on. Samples were included in analyses of cycle lengths, the bout length rule and transition preferences only if the number of recorded song type bouts was greater than twice the subject's repertoire size.

Field-recorded Males

We compared syntax in the two cohorts of hand-reared birds to syntax in 21 male song sparrows recorded in the field as previously described (Searcy et al., 2022). Briefly, these birds were recorded in Crawford County, Pennsylvania, U.S.A. during May and June of 2019. We recorded each subject twice with a mean of 9.5 days (range 5–14) between recording sessions. These birds were recorded using digital recorders (Marantz PMD 660 or 670) and cardioid microphones (Shure SM58) in parabolic reflectors (Sony PBR-330) at a sampling rate of 44.1 or 48 kHz. We recorded on average 345 songs per individual (range 295–500) during the first recording session and 316 songs (range 288–383) during the second. For all 21 subjects, all the song types recorded in the first session were also recorded in the second session and vice versa, giving us confidence that we recorded the full repertoires and one or more full cycles in each recording session.

Analysis

Song type identification

For the two groups of captive-reared birds, we assigned songs to song types by visual inspection of spectrograms made using Syrinx software with either a Blackman or a Hanning window and a 512-point fast Fourier transform (FFT). For the field-recorded males, spectrograms were made using Audacity software (http://www. audacityteam.org) with a Hanning window and a 256-point FFT. We classified two songs as the same song type if they shared the same introductory phrase and half or more of all phrases. Spectrograms of one or more renditions of each song type were printed out to aid in classification. Although song sparrows vary renditions of the same song type (Stoddard et al., 1988), within-type differences are smaller than between-type differences (Podos et al., 1992) and elicit lower dishabituation responses from receivers (Searcy et al., 1995, 1999). In previous work in this study population, observers blindly classifying songs to song types agreed on the correct classification in 97.7% of cases (Searcy et al., 2019).

Eventual variety rule

To test the eventual variety rule, we calculated the mean bout length for each subject and the proportion of bouts consisting of a single song. For both sets of estimates, only bouts known to be fully recorded were included. For the trained birds, analysis started with the first full bout after lights on and excluded the last bout recorded, which may not have been fully captured. For isolate birds, analysis excluded both the first and last bouts recorded. The eventual variety rule predicts that mean bout lengths will be substantially greater than 1 and that bouts consisting of only a single song will be rare.

Cycling rule

To test the cycling rule, we determined for each recording session the initial cycle length, where cycle length is the number of song type bouts a male uses to produce all the song types in his repertoire. Analysis of initial cycle lengths started with the first song recorded in a session for isolate birds and with the first song recorded after lights on for the trained birds. Minimum cycle length is equal to the subject's repertoire size. We compared observed cycle lengths to mean cycle lengths found in random sequences generated using song types drawn with replacement from repertoires of the same size as the subject's (see Searcy et al., 2022). We tested whether observed cycle lengths were less than those in random sequences using sign tests. The cycling rule predicts that observed cycle lengths will be lower than mean cycle lengths from random sequences and that cycles of minimum length will be common.

Bout length rule

We tested the prediction of the bout length rule that the length of a song bout will be positively correlated with length of the subsequent recurrence interval. Recurrence intervals are measured here as the number of bouts of other song types that are produced between two bouts of a focal song type (Hedley, 2016; Kroodsma, 1975). For each recording session, we measured bout length and subsequent recurrence interval for the first full bout of each song type in each recording session (Searcy et al., 2022). We combined the data for the two recording sessions within a year for each subject and calculated the Pearson's correlation coefficient between bout length and subsequent recurrence interval. We then tested whether the mean correlation for the sample of males was greater than 0 with a two-tailed one-sample t test.

Usage preferences

Usage refers to the number of copies of each song type produced by a male. Usage thus depends on both bout lengths and numbers of bouts. We tested for usage preferences by correlating usage of a male's song types in one sample of songs with usage of the same song types in a second sample. Positive correlations imply usage preferences that are stable over the period between the two song samples. We tested whether the mean correlation coefficient for a

sample of males was significantly greater than 0 using a two-tailed one-sample *t* test.

Transition preferences

We tested for transition preferences that were consistent within individuals from recording session to recording session following Hedley et al. (2018) and Searcy et al. (2022). The procedure involves (1) compiling a matrix of observed transitions between song types within each recording session for which the number of recorded transitions is greater than twice the repertoire size, (2) using one-tailed Fisher's exact tests to identify transitions within the matrix that occur more often than expected and (3) using a second one-tailed Fisher's exact test to determine whether the number of transitions that are preferred in both recording sessions for an individual within a year exceed random expectations.

To determine whether the trained subjects learned transition preferences from the training stimuli, we first determined which model songs they learned. Although male song sparrows in western populations often learn entire song types from tutors (Beecher et al., 1994; Nordby et al., 1999), song sparrows in eastern populations most often learn only part of any one song type (Marler & Peters, 1987, 1988). Nevertheless, in most cases we could determine from the sharing of notes and syllables which tutor song was the primary model for any one song type developed by a trained male. For 10 of the 13 trained males whose transitions we analysed, we were able to identify the model songs copied for all song types in their repertoires. For the remaining three males, we were able to identify the copied songs for all but one song type. Once the equivalencies between a subject's songs and model songs were found, we could determine for each transition in the male's recorded song sequences whether the transition was present in one or more of the 12 training sequences. We then tested the prediction that if first-order transition preferences are affected by learning, those transitions present in the training stimuli should be over-represented in the sequences recorded from the trained captives.

Ethical Note

All procedures for recording, hand-rearing and housing were approved by the Institutional Animal Care and Use Committee of Duke University (protocols A316-94-6R2 and A032-14-02). Field-recorded birds were recorded during naturally occurring singing, with no intervention or other kind of disturbance. Nestlings for the isolation and trained groups were collected from the wild under permits from the U.S. Fish and Wildlife Service, the North Carolina Wildlife Resources Commission and the Pennsylvania Game Commission. When collecting nestlings, whole nests were gently removed from the vegetation in which they were situated, wrapped in a cloth and transported within 30 min to the laboratory, where half-hourly feeding began immediately and continued from dawn until dusk until the birds fledged. As nestlings, birds were kept with their siblings in a natural song sparrow nest that had been sanitized through heating to eliminate potential pathogens and parasites. These nests were kept in plastic bins in a sound-attenuating room to minimize disturbance, in a facility designed and approved for songbird husbandry, with a seasonally varying daylength that matched their natural annual cycle. As adults, birds were housed in individual cages in the same approved facility. Birds were provided with a regular rotation of enrichments, such as special food stuffs and water baths. Data from the trained birds and isolate birds were obtained from earlier studies, meaning that no additional individuals were hand-reared for the present study.

RESULTS

Eventual Variety Rule

Figure 1 shows examples of two song sequences from one isolate male. This male adhered strongly to the eventual variety pattern as did the isolate males as a whole: on average (±SE), the isolates sang 14.9 ± 2.11 songs per bout (Fig. 2a) and produced single-song bouts on average in only 0.068 of total bouts (Fig. 2b). These values are similar to those from the field-recorded males, which had slightly shorter bout lengths (mean $= 14.0 \pm 1.04$ songs; Fig. 2a) and a slightly lower proportion of single-song bouts (mean $= 0.031$; Fig. 2b).

Although the trained males also followed the eventual variety rule (see Fig. 1 for an example), their overall mean bout lengths (6.9 ± 1.05 songs) were significantly shorter than those of the field-recorded birds ($t_{36} = 4.766$, $P < 0.001$; Fig. 2a), while their proportion of single-song bouts (mean $= 0.22$) was significantly higher (Mann–Whitney U test: $U = 68$, $z = -3.260$, two-tailed $P < 0.001$; Fig. 2b). These differences, however, might be explained by the low number of songs observed in some of the recording sessions for the trained males, as bout lengths tend to be low if birds are singing only occasionally. When we confined the analysis to recording sessions in which the numbers of songs were as large as those in the field-recorded samples ($N > 287$ songs), mean bout length for the trained birds (12.0 ± 1.26) (Fig. 2a) was not significantly different from that for the field-recorded males ($t_{26} = 1.036$, $P = 0.310$) and the proportion of bouts consisting of a single song (0.016) (Fig. 2b) was lower, although not significantly so (Mann–Whitney U test: $U = 48$, $z = -1.378$, $P = 0.168$).

Cycling Rule

The sequences in Fig. 1 illustrate how both isolate and trained males often string together successive cycles of minimum or close to minimum cycle lengths. Calculating average cycle length based on an entire sequence, however, may introduce bias because long cycles towards the end of the sequence are less likely to be completed and so may be differentially dropped from calculations of the mean. To avoid this bias, we calculated mean cycle lengths using just the initial cycle in each recording session. The four isolate male song sparrows in our sample had small repertoire sizes (Appendix, Table A1), ranging from three to six song types, and correspondingly small expected cycle lengths. Mean initial cycle lengths (averaged over the two recording sessions) were smaller than expected for three of four subjects and equal to the expected in the fourth (Fig. 2, Appendix, Table A1), but the sample of males was too small for a difference between observed and expected to be significant (by a sign test) even if all the observed means were lower than the expected means. It is nevertheless interesting to note that the observed initial cycle lengths took the minimum possible value in five of eight recording sessions across the four subjects (62.5%). By comparison, observed initial cycle lengths in field-recorded males took their minimum values in 26 of 42 recording sessions (61.9%).

For the trained males as a group, initial cycle lengths were lower than expected in each of the 3 years of recording (Fig. 3, Appendix, Table A2). The departure from random expectation was significant in each of the 3 years according to two-tailed sign tests (2014: $z = 3.051$, $N = 13$, $P = 0.0023$; 2015: $z = 3.464$, $N = 12$, $P = 0.00053$; 2016: $z = 3.606$, $N = 13$, $P = 0.00031$). Initial cycle lengths were the minimum possible in 48 of 71 recording sessions (67.6%). Just one male in one year had an observed mean cycle length that exceeded

Isolate male 302 – Session 1

$38C\rightarrow6B\rightarrow5D\rightarrow33E\rightarrow29A\rightarrow20C\rightarrow8B\rightarrow4D\rightarrow23E\rightarrow2A$

Isolate male 302 – Session 2

$12A\rightarrow11B\rightarrow6D\rightarrow20E\rightarrow5C\rightarrow11A\rightarrow SB\rightarrow7C\rightarrow1E\rightarrow4B\rightarrow3D\rightarrow$
$24A\rightarrow10E\rightarrow8C\rightarrow9B\rightarrow4D\rightarrow 6B\rightarrow14C\rightarrow2A$

Trained male 724 – Session 1 (2014)

$3D\rightarrow2F\rightarrow10B\rightarrow6A\rightarrow9C\rightarrow7D\rightarrow12E\rightarrow14F\rightarrow30B\rightarrow14C \rightarrow11D\rightarrow12E\rightarrow4A\rightarrow$
$2F\rightarrow7B\rightarrow15C\rightarrow12E\rightarrow5D\rightarrow12F\rightarrow5A\rightarrow15B\rightarrow13C\rightarrow7D\rightarrow3E\rightarrow9A\rightarrow8F\rightarrow$
$2D\rightarrow16E\rightarrow8B\rightarrow12C\rightarrow2F\rightarrow13D\rightarrow8A\rightarrow9B\rightarrow7F\rightarrow10E\rightarrow14C\rightarrow7B\rightarrow8A\rightarrow8E\rightarrow6D\rightarrow$
$6F\rightarrow7C\rightarrow14B\rightarrow3E\rightarrow7F\rightarrow15D\rightarrow5E\rightarrow9A\rightarrow8C\rightarrow7F$

Trained male 724 – Session 2 (2014)

$10A\rightarrow10B\rightarrow8E\rightarrow11F\rightarrow12C\rightarrow10D\rightarrow10E\rightarrow11A\rightarrow9B\rightarrow11F\rightarrow8D\rightarrow12C\rightarrow$
$13E\rightarrow2B\rightarrow11A\rightarrow10F\rightarrow8D\rightarrow13B\rightarrow12C\rightarrow9E\rightarrow5D\rightarrow10F\rightarrow6A\rightarrow12B\rightarrow12C\rightarrow$
$6E\rightarrow5D\rightarrow5F\rightarrow6A\rightarrow8B\rightarrow14E\rightarrow11C\rightarrow5F\rightarrow9D\rightarrow5A\rightarrow3B\rightarrow6F\rightarrow7E\rightarrow8C\rightarrow$
$9B\rightarrow8A\rightarrow3F\rightarrow7D\rightarrow1E\rightarrow4C\rightarrow1F$

Figure 1. Song sequences for one isolate male and one trained male. For both, the two sequences analysed for 1 year are shown. Boxes delineate successive cycles, with box colour coding cycle length: red for the minimum length given the repertoire sizes (5 song types for the isolate male, 6 for the trained male), blue for one more than the minimum, and light green for two more than the minimum. All the observed cycle lengths were well below the expected lengths of 9.3 bouts for a repertoire of five song types and 12.4 bouts for a repertoire of six song types.

random expectation (male 704 in 2014) (Fig. 3). This outlier might be explained by the fact that this male was in the midst of dropping three of his 11 song types during his first year of recording; in the two succeeding years he sang only eight song types. Rare song types are expected to increase cycle lengths. Only one other male in our sample dropped any song types from one year to the next: male 735 dropped one song type between 2014 and 2015, and this bird had the second highest mean cycle length in 2014 (although still shorter than random expectation).

Bout Length Rule

The bout length rule predicts a positive correlation between the length of a song type bout and the length of the subsequent recurrence interval, i.e. the interval until that song type is sung again. The four isolate males showed no evidence for such a relationship: the mean Pearson correlation between bout length and subsequent recurrence interval for these birds was -0.018 ± 0.237 (Fig. 2c). For the trained males, the correlations between bout length and recurrence interval were mainly positive (Appendix, Table A3). The mean correlation was significantly greater than 0 by a two-tailed one-sample t test in 2015 ($t_{10} = 4.404$, $P < 0.001$) and 2016 ($t_{11} = 2.779$, $P = 0.018$). The mean correlation in 2014 was positive but not significantly greater than 0 ($t_9 = 2.238$, $P = 0.052$). The mean of the per-male mean correlations was 0.271 ± 0.075 (Fig. 2c), which was significantly greater than 0 by a two-tailed one-sample t test ($t_{12} = 3.585$, $P = 0.004$). Although these mean correlations for the trained birds were significantly positive, they were lower than the bout length/recurrence interval correlations for the field-recorded birds (mean $r = 0.485 \pm 0.058$; Fig. 2c). The difference between the trained and field-recorded birds was significant by a two-tailed two-sample t test ($t_{32} = 2.253$, $P = 0.031$).

Usage Preference Rule

Our tests for usage preferences entailed correlating a subject's song type usage frequencies in two separate recording sessions; if

such correlations are consistently positive, then that constitutes evidence of stable usage preferences over the corresponding time interval. For the four isolate males, the correlations in usage frequency between recording sessions 1 and 2 were -0.026, 0.985, 0.332 and 0.939, giving a mean ($\pm$SE) of 0.558 ± 0.245 (Fig. 2d). For the trained males, the mean correlation in usage frequencies between recording sessions was positive in all 3 years and significantly different from 0 in 2016 ($t_{11} = 6.682$, $P < 0.0001$) but not in 2014 ($t_9 = 1.851$, $P = 0.097$) and 2015 ($t_{10} = 1.780$, $P = 0.106$). The individual mean correlations averaged over the 3 years were also significantly greater than 0 ($t_{12} = 5.360$, $P < 0.0001$; Appendix, Table A4). Comparing usage across adjacent years, mean correlations (Appendix, Table A4) were significantly greater than 0 both for the 2014–2015 comparison ($t_7 = 3.127$, $P = 0.017$) and for the 2015–2016 comparison ($t_9 = 3.524$, $P = 0.006$). The between-session within-year usage correlations for field-recorded males (mean $= 0.312$, range -0.339–0.948) were not significantly different from the mean between-session correlations for the trained group ($t_{32} = -1.063$, $P = 0.329$).

Transition Preferences

All four isolate birds met our sample size criteria for analysing transition preferences. Just one of these birds exhibited a consistently preferred transition (i.e. a specific transition that was overrepresented in both of its recording sessions); one consistently preferred transition was not more than expected by chance for this bird. Of the 13 trained males that met the sample size criteria in at least one year, eight had one or more transitions (range 1–3) that were consistently preferred across the two recording sessions within a year (Appendix, Table A5). Only one of these males, however, had significantly more consistently preferred transitions than expected by chance. This one subject (male 704) had three transitions that were preferred in both recording sessions in 2014, none in 2015 and two in 2016. The transitions that were consistently preferred in 2014 by this male (C–D, E–I, G–A) did not overlap with the transitions consistently preferred in 2016 (C–A, H–E).
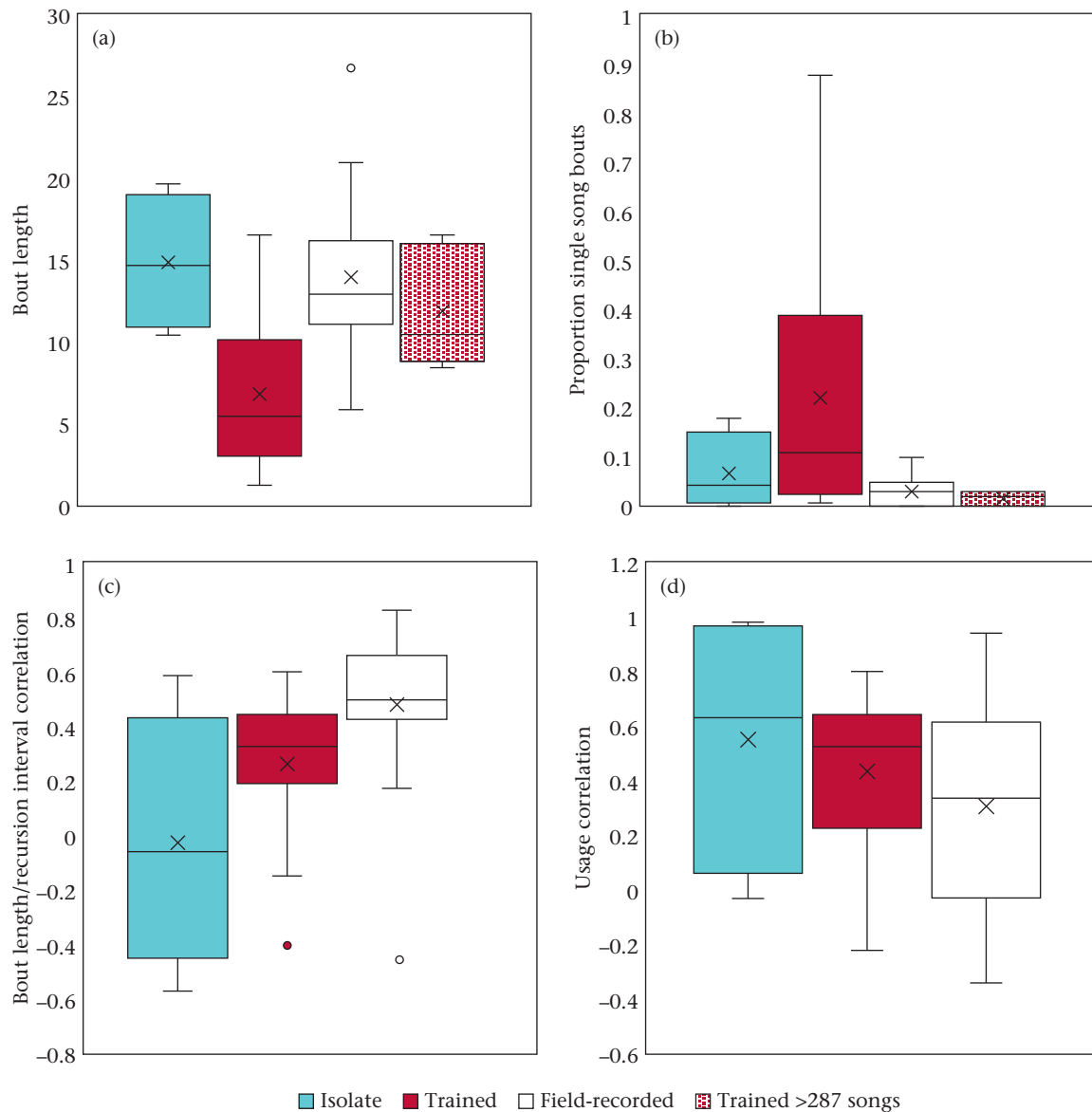
Figure 2. Box and whisker plots for (a) bout length for the three treatment groups (isolate, trained and field-recorded) plus the subset of trained birds with more than 287 recorded songs, (b) proportion of single song bouts for the same four groups, (c) bout length/recursion interval correlations for isolate, trained and field-recorded birds and (d) usage correlations for the same three groups. The box and whisker plots on one side of the median (centre line) show the first and second quartile of the data in that direction. The × shows the mean value.

Field-recorded birds showed similarly low levels of transition preferences. These males cycled through their repertoires using song type transitions that varied widely from cycle to cycle and from recording session to recording session (Searcy et al., 2022). Only two of the 21 field-recorded males exhibited one or more transitions that were more common than expected in both their recording sessions within a year.

The training sequences in aggregate contained 317 of the 992 transitions possible among the 32 tutor song types, or 32.0%. The transitions from the training stimuli in turn constituted on average 35.2% of the transitions found in the recordings of the 13 trained males meeting sample size criteria. The difference between the observed percentage and the percentage expected by chance was not significant (one-sample t test: $t_{12} = 0.819$, $P = 0.317$). Of the 992 transitions, 49 (4.9%) occurred two or more times in the training stimuli. These transitions were slightly under-represented in the recordings of the captive trained subjects, occurring just 2.9% of the

time, but the difference from the expected was again not significant ($t_{12} = -1.906$, $P = 0.081$). The subjects did not favour transitions from the first training set; instead, the mean percentage from that set (10.6%) was no different from random expectation (8.3%; $t_{12} = 0.401$, $P = 0.695$).

DISCUSSION

Our results indicate little or no effect of learning from external models on the development of three of the syntactical rules examined here. The first of these is the eventual variety rule, which mandates that a singer repeats a given song type multiple times before switching to another type. In the present study, isolate males raised with no experience of hearing external song sequences followed the eventual variety pattern similarly to field-recorded males that presumably had extensive external experience with the pattern. The trained males were tutored with model sequences
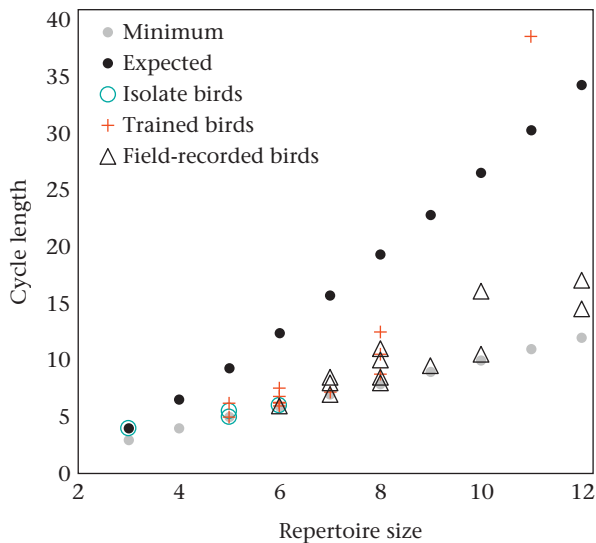
Figure 3. Cycle lengths for isolate, trained and field-recorded birds compared to expected values from random sequences and minimum values depending on repertoire size. The one observed value above expected (for a repertoire of 11) is a minimum value because the subject (trained male 704) did not finish his initial cycle during one recording period. The two trained males whose repertoire sizes changed between 2014 and 2015 are each represented by two points, one for their 2014 results and one for their 2015–2016 results.

that followed the eventual variety rule more strictly than sequences that song sparrows would experience in the field, with higher mean bout lengths and no single-song bouts at all, and yet they produced lower mean bout lengths than field-recorded birds. Thus, our results indicate that song sparrows default to the eventual variety pattern in the absence of the opportunity to learn from external models and are not pushed towards a more extreme eventual variety pattern by training with such a pattern. It is still possible, however, that training stimuli ordered with immediate variety might bias song sparrows towards immediate variety syntax, with shorter bout lengths and a higher proportion of single-song bouts.

A second rule that seems to be independent of external experience is the cycling rule, which states that a singer should cycle through its repertoire of song types efficiently, in close to the minimum number of bouts. The isolate males in this study, having no experience with external models of cycling, followed this rule convincingly, producing cycles of the minimum length with as high a frequency as the field-recorded birds. The trained group experienced a training regimen that should reinforce cycling, in that the training sequences included no short recurrence intervals in which a singer returned to a specific song type before singing all or most of its remaining repertoire. These trained birds also cycled with an efficiency similar to that of the field-recorded birds. Cycling thus develops with no training in song sparrows and seems resistant to improvement through training. We know of no previous studies of cycling development in songbirds. An interesting follow-up study would be to test whether song sparrows could be influenced to cycle less efficiently through training with random sequences of song types that explicitly do not follow the cycling rule. Another interesting follow-up would be to investigate the neural control of intersong syntax, as has been done for within-song syntax (Fujimoto et al., 2011).

The third syntactical rule that appears to be unaffected by learning from external models is the usage preference rule, which states that individuals consistently produce some of their song types more than others. Prior evidence for usage preferences in song sparrows comes from positive correlations between an individual's usage frequencies in different recording sessions (Searcy et al., 2022). In the present study, the isolate males, with no

external experience of usage patterns, showed usage correlations within breeding seasons that were on average at least as strong as in the field-recorded birds. The trained males were trained with model sequences in which usage frequencies were exactly equal across all song types; these birds again showed usage correlations between sessions within years that were at least as strong as in the field-recorded birds, and in addition showed significant usage correlations across years. Usage preferences thus emerged in hand-reared birds without external experience and seemed resistant to modification in response to training.

The one syntactical rule that was clearly modified by early experience was the bout length rule. The isolate birds did not show the positive correlations between bout length and subsequent recurrence interval predicted by the bout length rule. The trained group, tutored with sequences that did not follow the bout length rule, showed positive correlations between bout length and recurrence intervals, but these correlations were significantly weaker than in field-recorded males. The results from the two laboratory-reared groups were not entirely in accord, however, in that without any external experience of the bout length rule, isolate males produced no hint of the bout length rule, whereas the captive-trained males, tutored with sequences that should have reinforced the absence of any relationship between bout length and recurrence interval, actually produced a weakened version of the rule. Nevertheless, results from both groups suggest that the bout length rule is influenced at least to some extent by learning from external models.

Cycling in songbirds has been suggested to be produced by a mechanism in which inhibition of a focal song type builds up as that song is repeated in a bout, eventually causing the singer to switch away from that type (Falls, 1985; Hinde, 1958). Inhibition of the focal type is suggested to then dissipate gradually as the singer produces other song types. Once inhibition of the focal song is lower than inhibition of the competing song types, the singer returns to the focal song type (Hinde, 1958; Searcy et al., 2022). This hypothetical mechanism directly predicts the bout length rule as well as cycling (Searcy et al., 2022). Therefore, there is some difficulty in reconciling this inhibition/disinhibition mechanism with the present results: if the mechanism is working to produce cycling in isolate male song sparrows, why does it not also produce positive correlations between bout length and recurrence intervals? One possibility is that song sparrows possess some other simple mechanism that produces cycling but does not produce the bout length rule, although it is difficult to imagine what such a mechanism could be. Another possibility is that song sparrows do not use any kind of simple mechanism to cycle, but instead maintain an explicit memory of all that they have sung over the preceding cycle and select the next song to sing based on that memory. The birds could then have an innate tendency to cycle while learning from external models to adjust cycling to produce the bout length rule.

The aspect of syntax development that has been best studied in songbirds is the development of first-order transition preferences. In experimental tests in which juvenile birds were trained with playback in captivity, first-order transitions occurring in the training sequences were disproportionately produced by the subjects in nightingales (Hultsch & Todt, 1992), marsh wrens (Kroodsma, 1979) and chaffinches (Riebel & Slater, 1999). In contrast, we found that song sparrows did not disproportionately copy transitions heard in training sequences. The sample size of subjects used in our analysis of transition preferences was larger than in any of the preceding studies. Sample sizes were particularly low in the chaffinch study (three individuals) and the marsh wren study (two individuals). Details of the training procedures varied widely between the studies, perhaps most importantly in the number of times subjects were exposed to specific transitions. In

the present study, males were played a specific training sequence twice daily for 1 week, for a total of 14 presentations of each of the transitions contained in each sequence. Some specific first-order transitions occurred in two to three of the training sequences and so were heard by subjects 28 or 42 times. None of these transitions appeared to be learned disproportionately by the subjects as a whole. By contrast, subjects were played specific training sequences hundreds of times in the chaffinch study (Riebel & Slater, 1999) and thousands of times in the marsh wren study (Kroodsma, 1979), so greater exposure might explain why learning occurred in these species. Nightingales, however, showed evidence of copying transitions with as little as a single exposure to a training sequence. Moreover, the behavior of our trained subjects in not learning transitions from the training sequences is consistent with the general rarity of strong first-order transition preferences both in our trained captive group and in field-recorded song sparrows (Searcy et al., 2022). A valuable follow-up study would be to train hand-reared song sparrows with a smaller number of transitions each presented many more times.

Vocal production learning, in which the structure of individual vocalizations is learned, is a rare trait among animals, known only from a few scattered taxa of birds and mammals (Janik & Slater, 2000; Petkov & Jarvis, 2012; Searcy & Nowicki, 2019). By contrast, vocal usage learning, in which an animal learns the context in which to produce particular vocalizations, is thought to be more widespread (Janik & Slater, 1997, 2000). In song sparrows, however, the structure of songs is known to be profoundly affected by early learning (Beecher et al., 1994; Kroodsma, 1977; Nordby et al., 2000; Nowicki et al., 2002), whereas we have now shown that most aspects of between-song syntax, all of which are components of usage, are not learned in this way. A challenge for future research is to understand why some aspects of intersong syntax are open to the effects of learning and others are not.

Author Contributions

Acknowledgments

References

Anderson, R. C., Searcy, W. A., Peters, S., Hughes, M., DuBois, A. L., & Nowicki, S. (2017). Song learning and cognitive ability are not consistently related in a songbird. *Animal Cognition, 20*, 309–320.

Beecher, M. D., Campbell, S. E., & Stoddard, P. K. (1994). Correlation of song learning and territory establishment strategies in the song sparrow. *Proceedings of the National Academy of Sciences of the U.S.A., 91*, 1450–1454.

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

Doupe, A. J., & Kuhl, P. K. (1999). Birdsong and human speech: Common themes and mechanisms. *Annual Review of Neuroscience, 22*, 567–631.

Falls, J. B. (1985). Song matching in western meadowlarks. *Canadian Journal of Zoology, 63*, 2520–2524.

Fujimoto, H., Hasegawa, T., & Watanabe, D. (2011). Neural coding of syntactic structure in learned vocalizations in the songbird. *Journal of Neuroscience, 31*, 10023–10033.

Hartshorne, C. (1956). The monotony-threshold in singing birds. *Auk, 73*, 176–192.

Hedley, R. W. (2016). Complexity, predictability and time homogeneity of syntax in the songs of Cassin's vireo (*Vireo cassinii*). *PLoS One, 11*, Article e0150822.

Hedley, R. W., Logue, D. M., Benedict, L., & Mennill, D. J. (2018). Assessing the similarity of song-type transitions among birds: Evidence for interspecies variation. *Animal Behaviour, 140*, 161–170.

Hinde, R. A. (1958). Alternative motor patterns in chaffinch song. *Animal Behaviour, 6*, 211–218.

Hultsch, H. (1991). Early experience can modify singing styles: Evidence from experiments with nightingales, *Luscinia megarhynchos. Animal Behaviour, 42*, 883–889.

Hultsch, H., & Todt, D. (1992). The serial order effect in the song acquisition of birds: Relevance of exposure frequency to song models. *Animal Behaviour, 44*, 590–592.

Hultsch, H., & Todt, D. (1996). Discontinuous and incremental processes in the song learning of birds: Evidence for a primer effect. *Journal of Comparative Physiology, 179*, 291–299.

Janik, V. M., & Slater, P. J. B. (1997). Vocal learning in mammals. *Advances in the Study of Behavior, 26*, 59–99.

Janik, V. M., & Slater, P. J. B. (2000). The different roles of social learning in vocal communication. *Animal Behaviour, 60*, 1–11.

Kroodsma, D. E. (1975). Song patterning in the rock wren. *Condor, 77*, 294–303.

Kroodsma, D. E. (1977). A re-evaluation of song development in the song sparrow. *Animal Behaviour, 25*, 390–399.

Kroodsma, D. E. (1979). Vocal dueling among male marsh wrens: Evidence for ritualized expressions of dominance/subordinance. *Auk, 96*, 506–515.

Lapierre, J. M., Mennill, D. J., & MacDougall-Shackleton, E. A. (2011). Spatial and age-related variation in use of locally common song elements in dawn singing of song sparrows *Melospiza melodia*: Old males sing the hits. *Behavioral Ecology and Sociobiology, 65*, 2149–2160.

Leitner, S., & Catchpole, C. K. (2007). Song and brain development in canaries raised under different conditions of acoustic and social isolation over two years. *Developmental Neurobiology, 67*, 1478–1487.

Lipkind, D., Marcus, G. F., Bemis, D. K., Sasahara, K., Jacoby, N., Takahashi, M., Suzuki, K., Feher, O., Ravbar, P., Okanoya, K., & Tchernichovski, O. (2013). Stepwise acquisition of vocal combinatorial capacity in songbirds and human infants. *Nature, 498*, 104–109.

Marler, P. (1970a). A comparative approach to vocal learning: Song development in white-crowned sparrows. *Journal of Comparative & Physiological Psychology, 71*, 1–25.

Marler, P. (1970b). Birdsong and speech development: Could there be parallels? *American Scientist, 58*, 669–673.

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

Marler, P., & Peters, S. (1988). The role of song phonology and syntax in vocal learning preferences in the song sparrow, *Melospiza melodia. Ethology, 77*, 125–149.

Marler, P., & Sherman, V. (1985). Innate differences in singing behaviour of sparrows reared in isolation from adult conspecific song. *Animal Behaviour, 33*, 57–71.

Nice, M. M. (1943). Studies in the life history of the song sparrow. II. The behavior of the song sparrow and other passerines. *Transactions of the Linnaean Society of New York, 6*, 1–328.

Nordby, J. C., Campbell, S. E., & Beecher, M. D. (1999). Ecological correlates of song learning in song sparrows. *Behavioral Ecology, 10*, 287–297.

Nordby, J. C., Campbell, S. E., & Beecher, M. D. (2002). Adult song sparrows do not alter their song repertoires. *Ethology, 108*, 39–50.

Nordby, J. C., Campbell, S. E., Burt, J. M., & Beecher, M. D. (2000). Social influences during song development in the song sparrow: A laboratory experiment simulating field conditions. *Animal Behaviour, 59*, 1187–1197.

Nowicki, S., Searcy, W. A., & Peters, S. (2002). Quality of song learning affects female response to male bird song. *Proceedings of the Royal Society, Series B: Biological Sciences, 269*, 1949–1954.

Petkov, C. I., & Jarvis, E. D. (2012). Birds, primates, and spoken language origins: Behavioral phenotypes and neurobiological substrates. *Frontiers in Evolutionary Neuroscience, 4*, 12. https://doi.org/10.3389/fnevo.2012.00012

Podos, J., Peters, S., Rudnicky, T., Marler, P., & Nowicki, S. (1992). The organization of song repertoires in song sparrows: Themes and variations. *Ethology, 90*, 89–106.

Riebel, K., & Slater, P. J. B. (1999). Do male chaffinches *Fringilla coelebs* copy song sequencing and bout lengths from their tutors? *Ibis, 141*, 680–686.

Saunders, A. A. (1924). Recognizing individual birds by song. *Auk, 41*, 242–259.

Searcy, W. A., & Nowicki, S. (2019). Birdsong learning, avian cognition and the evolution of language. *Animal Behaviour, 151*, 217–227.

Searcy, W. A., Nowicki, S., & Peters, S. (1999). Song types as fundamental units in vocal repertoires. *Animal Behaviour, 58*, 37–44.

Searcy, W. A., Ocampo, D., & Nowicki, S. (2019). Constraints on song type matching in a songbird. *Behavioral Ecology and Sociobiology, 73*, 102. https://doi.org/10.1007/s00265-019-2708-6

Searcy, W. A., Podos, J., Peters, S., & Nowicki, S. (1995). Discrimination of song types and variants in song sparrows. *Animal Behaviour, 49*, 1219–1226.

Searcy, W. A., Soha, J., Peters, S., & Nowicki, S. (2022). Long-distance dependencies in birdsong syntax. *Proceedings of the Royal Society B: Biological Sciences, 289*, Article 20212473. https://doi.org/10.1098/rspb2021.2473

Soha, J. A., & Peters, S. (2015). Vocal learning in songbirds and humans: A retrospective in honor of Peter Marler. *Ethology, 121*, 933–945.

Stoddard, P. K., Beecher, M. D., & Willis, M. S. (1988). Response of territorial male song sparrows to song types and variations. *Behavioral Ecology and Sociobiology, 22*, 125–130.

Thorpe, W. H. (1958). The learning of song patterns by birds, with especial reference to the song of the chaffinch *Fringilla coelebs. Ibis, 100*, 535–570.

Todt, D., & Hultsch, H. (1996). Acquisition and performance of song repertoires: Ways of coping with diversity and versatility. In D. E. Kroodsma, & E. H. Miller (Eds.), *Ecology and evolution of acoustic communication in birds* (pp. 79–96). Ithaca, New York: Cornell University Press.

Todt, D., & Hultsch, H. (1998). How songbirds deal with large amounts of serial information: Retrieval rules suggest a hierarchical song memory. *Biological Cybernetics, 79*, 487–500.

Appendix

Table A1
Initial cycle lengths of the isolate males

Male ID	Repertoire size	Session 1			Session 2			Mean cycle length	Expected cycle length
		Song no.	Switches	Cycle length	Song no.	Switches	Cycle length		
300	6	192	12	**6**	180	10	**6**	6	12.4
301	3	172	10	4	168	8	4	4	4.0
302	5	168	9	**5**	162	18	**5**	5	9.3
303	5	110	12	**5**	132	12	6	5.5	9.3

Bold indicates a cycle of minimum length given the individual's repertoire size.

Table A2
Initial cycle lengths of the trained males

Male ID	Repertoire size	Expected cycle length	2014		2015		2016	
			Cycle lengths	Mean	Cycle lengths	Mean	Cycle lengths	Mean
704	11 (8)	30.2 (19.3)	21, ≥55	≥ 38	**8, 8**	8	9, 10	9.5
707	7	15.7	**7, 7**	7	**7, 7**	7	8, **7**	7.5
712	6	12.4	**6**, 7	6.5	**6, 6**	6	**6, 6**	6
714	6	12.4	—, **6**	6	7, **6**	6.5	10, **6**	8
716	6	12.4	—, —	—, —	—, —	—, —	9, **6**	7.5
717	5	9.3	**5**, —	5	—, —	—, —	—, —	—, —
720	5	9.3	—, 8	8	**5**, —	5	**5**, 6	5.5
722	7	15.7	8, **7**	7.5	8, **7**	7.5	**7, 7**	7
723	6	12.4	**6, 6**	6	**6, 6**	6	**6, 6**	6
724	6	12.4	7, **6**	6.5	7, **6**	6.5	**6, 6**	6
726	6	12.4	**6, 6**	6	7, **6**	6.5	**6, 6**	6
732	6	12.4	7, **6**	6.5	9, **6**	7.5	7, **6**	6.5
734	8	19.3	**8, 8**	8	**8**, 17	12.5	—, 11	11
735	8 (7)	19.3 (15.7)	17, **8**	12.5	**7, 7**	7	**7**, 8	7.5

Two cycle lengths under each year are the initial cycle lengths for the two recording sessions. Where two repertoire sizes are given, the first applies to 2014 and the second to 2015 and 2016. Bold indicates a cycle of minimum length given the individual's repertoire size. A dash indicates that no cycle length was estimated because the sample size of transitions was under criterion.

Table A3
Correlations between bout length and subsequent recurrence interval for the trained captive group

Male ID	Correlation			Mean correlation
	2014	2015	2016	
704	−0.308	−0.116	−0.006	−0.143
707	0.372	0.000	0.484	0.285
712	0.494	0.351	−0.231	0.205
714	—	0.723	0.495	0.609
716	—	—	0.515	0.515
720	—	—	−0.396	−0.396
722	0.298	0.618	0.332	0.416
723	0.558	0.449	0.448	0.485
724	−0.095	0.705	0.396	0.335
726	0.206	0.268	0.636	0.370
732	0.338	0.148	0.439	0.308
734	0.313	0.373	—	0.343
735	−0.139	0.645	0.052	0.186
Mean	0.204	0.378***	0.264*	0.271**

Values are missing for male/years in which numbers of recorded transitions were below our criteria. *P < 0.05; **P < 0.01; ***P < 0.001.

Table A4
Correlations for the trained captive group between song type usage frequencies in the two recording sessions within a year and between two successive years

Male ID	Repertoire size	Correlation within years			Mean correlation	Correlation between years	
		2014	2015	2016	2014–2016	2014 vs 2015	2015 vs 2016
704	11 (8)	0.920	0.613	0.570	0.701	—	0.390
707	7	−0.269	0.073	0.762	0.189	0.503	0.794
712	6	−0.466	0.833	0.925	0.431	0.670	0.715
714	6	—	0.000	0.736	0.368	—	−0.071
716	6	—	—	0.588	0.588	—	—
720	5	—	—	0.807	0.807	—	—
722	7	0.101	0.396	−0.209	0.096	0.084	0.923
723	6	0.889	−0.520	0.446	0.272	0.620	−0.394
724	6	0.753	0.273	0.752	0.592	0.861	0.794
726	6	−0.589	−0.483	0.423	−0.216	-0.422	0.325
732	6	0.618	0.839	0.957	0.805	0.891	0.974
734	8	0.791	0.280	—	0.536	0.833	—
735	8 (7)	0.732	0.391	0.468	0.530	—	0.521
mean		.348	.245	0.602***	0.438***	0.505*	0.497**

Where two values are given for repertoire size, the first is for 2014 and the second is for 2015–2016. A dash indicates no correlation was calculated because one sample or both samples were below our size criterion or because repertoire size changed between years. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table A5
Consistently preferred transitions in the recordings of the trained captive group

Male ID	Year	Song repertoire size	SS(1)	SS(2)	Consistently preferred transitions	Obs/Exp	P
704	2014	11	44	54	3	6.875	0.005
	2015	8	25	38	0	0	1
	2016	8	41	37	2	5.6	0.036
707	2014	7	33	42	0	0	1
	2015	7	34	34	1	7	0.139
	2016	7	33	32	1	4.667	0.204
712	2014	6	20	20	1	3.333	0.28
	2015	6	24	26	0	NA	1
	2016	6	26	31	0	NA	1
714	2015	6	14	19	0	0	1
	2016	6	34	41	0	NA	1
716	2016	6	37	19	0	0	1
720	2016	5	11	24	0	NA	1
722	2014	7	50	39	0	0	1
	2015	7	43	36	0	0	1
	2016	7	36	38	1	10.5	0.095
723	2014	6	27	30	1	15	0.067
	2015	6	19	29	0	NA	1
	2016	6	23	29	0	NA	1
724	2014	6	50	45	0	0	1
	2015	6	46	38	0	0	1
	2016	6	46	53	1	7.5	0.133
726	2014	6	25	24	0	0	1
	2015	6	18	16	0	0	1
	2016	6	22	20	0	0	1
732	2014	6	23	31	0	NA	1
	2015	6	33	32	0	NA	1
	2016	6	27	31	0	0	1
734	2014	8	50	46	1	4.667	0.202
	2015	8	23	33	0	0	1
735	2014	8	29	33	1	18.667	0.054
	2015	7	25	31	0	NA	1
	2016	7	35	39	1	4.2	0.226

SS(1) and SS(2) are sample sizes of observed transitions in the first and second recording sessions, respectively. NA indicates that an observed/expected ratio could not be calculated because the denominator was 0 (due to the absence of any preferred transitions in one or both recording sessions).