Song ontogeny in Nuttall's white-crowned sparrows tutored with individual phrases

Jill A. Soha*

Department of Biology, Duke University, Box 90338, Durham, NC, 27708, United States

A R T I C L E   I N F O

A B S T R A C T

Behavioral ontogeny involves the interaction of innate predispositions and experience. In bird song learning, one approach to exploring this interaction is to examine the songs rehearsed by young birds whose exposure to tutor models has been carefully controlled. Here, I analyzed the rehearsed repertoire in Nuttall's white-crowned sparrows (*Zonotrichia leucophrys nuttalli*) tutored with individual phrases of conspecific and heterospecific songs. The proportions of phrase types rehearsed indicate that the learning biases evident in crystallized song are manifest early on, suggesting preferential memorization rather than preferential retention during attrition. The proportion of songs beginning with whistles increased during song rehearsal and phrase sequence variability decreased, consistent with the idea that innate syntax specifications guide song rehearsal. Single-phrase tutored birds overproduced phrases to the same extent previously observed in birds tutored with full, normal song but retained fewer phrase types in their crystallized repertoires. This suggests that in this subspecies, acquired syntax information does not affect the number of phrase types memorized and rehearsed but does affect repertoire attrition at the end of the sensorimotor phase. I discuss these results with a focus on the action of innate templates in song development and subspecies differences in this process.

1. Introduction

Bird song learning has been studied in detail in a number of species, and many aspects of this developmental process and how it varies across species are well understood (Catchpole and Slater, 2008). In almost all songbird species studied to date, young birds must hear adult conspecific song in order to develop normal songs themselves. The sensory phase during which these songs are heard (and memorized) is followed by a sensorimotor phase during which the young bird rehearses his own vocalizations, gradually matching them to an internal model using auditory feedback. In many species, including new world sparrows (Emberizidae), this internal model closely resembles the memorized conspecific songs, resulting in the persistence of song types from one generation to the next. At the end of the sensorimotor phase, a subset of the rehearsed song elements become crystallized into the adult repertoire and are produced in a stereotyped manner, consistently from rendition to rendition.

The interaction of innate predispositions and experience is evident throughout this developmental process. Multiple-choice song tutoring experiments have shown that birds preferentially attend to and learn conspecific song (Thorpe, 1958; Marler and Tamura, 1964; Soha et al., 2009). Isolation experiments have shown that without external input,

most birds develop songs that lack some normal species-typical features (e.g., Thorpe, 1958; Immelmann, 1969; Marler and Sherman, 1985). In closed-ended learners, which acquire new songs only when young, the timing of this sensory phase depends on both internal and external factors (Kroodsma and Pickert, 1980; Slater et al., 1988; Doupe and Kuhl, 1999). These observations come from studies of songs produced in adulthood by birds raised in controlled conditions. Studying the process of song development itself, by examining the vocal output of young birds (plastic song) and how it changes during the sensorimotor stage, provides additional information about how predispositions and experience interact in song ontogeny. For example, such within-subjects longitudinal studies have documented overproduction (the rehearsal of excess material) followed by selective repertoire attrition (Marler and Peters, 1982a) that is shaped by both external (Marler and Nelson, 1993) and internal factors (Soha, 2017).

In addition to the analysis of plastic song, another useful tool for studying song development is the use of specially modified stimuli in song tutoring experiments. Assessing what birds learn from carefully designed sets of tutor songs or phrases can provide novel, detailed information about selective learning. For example, the relative importance of species-typical phonology (note structure) and song syntax (phrase number and sequencing) in guiding selective song learning has

---

been elucidated for swamp sparrows *Melospiza georgiana* (Marler and Peters, 1977), song sparrows *Melospiza melodia* (Marler and Peters, 1988), and white-crowned sparrows *Zonotrichia leucophrys* (Soha and Marler, 2000) by tutoring these birds with natural song components recombined in particular ways. Tutoring with individual phrases can yield information about whether young birds learn certain phrase types more readily than others and how song syntax arises during development (Soha and Marler, 2001a).

Here, I combined the two approaches of modified-song tutoring and plastic song analysis to further study the process of song development in the white-crowned sparrow. I exposed young males of the subspecies *Z. l. nuttalli* to a set of individually presented single phrases, including whistles, buzzes and trills taken from conspecific and heterospecific songs. I then analyzed the vocalizations produced by these tutored birds at three stages. I examined the changes across stages and compared the rehearsed repertoire both to the set of tutor phrases and to the songs that the birds crystallized, to address the following three questions.

First, how does the relative salience of different phrase types change as song development proceeds? Prior to tutoring, fledgling white-crowned sparrows respond equally to all conspecific phrase types (whistles and non-whistles), and they also respond preferentially to conspecific phrases over heterospecific ones regardless of syntax (Whaling et al., 1997; Soha and Marler, 2001b). However, analysis of the songs learned in tutoring experiments indicates that white-crowned sparrows preferentially learn whistles over other phrase types but in the absence of key syntactical information they exhibit no bias towards learning conspecific phrases over heterospecific ones (Soha and Marler, 2000, 2001a). Distinct patterns of phrase preference are therefore evident before and after the process of song learning. How does this occur? One possibility is that song (phrase) memorization mirrors the selective response pattern observed in fledglings, such that conspecific phrases (but not whistles) are preferentially memorized, and the alternate pattern seen in crystallized song then arises at the end of the sensorimotor phase through selective retention of whistles and any heterospecific phrases. Another possibility is that the pattern of selectivity seen in crystallized songs arises earlier, during the sensory phase, such that whistles (rather than buzzes and trills) are preferentially memorized and rehearsed while conspecific and heterospecific phrases are memorized and rehearsed in equal proportions.

Understanding how phrase type salience changes over development is relevant for further understanding how auditory templates, which are representations of song in the bird's neural circuitry, guide song learning in this species. (Marler, 1984 and Marler, 1997) postulated that innate auditory templates are of two types: latent templates, which require activation by external input before they can contribute to the process, and preactive templates, which do not. Normally, both work together to facilitate memorization of appropriate song models to form the acquired template. If a bird hears no song when young, however, the latent template is not activated and the preactive template alone guides song development. In white-crowned sparrows, birds raised without tutoring sing primarily whistles but also develop aspects of normal syntax (phrase number and order), indicating that in this species, the preactive template specifies whistles and basic song syntax (Soha, 2017). The multiple non-whistle phrases typical of normal song in this species do not develop in acoustically isolated white-crowned sparrows, and according to Marler's model, these phrases therefore reflect the specifications of the latent template. As stated above, young fledglings preferentially respond to all conspecific phrases including non-whistles (buzzes, trills and note complexes), regardless of syntax. The fact that this occurs on first exposure, prior to song learning, suggests that it reflects activation of the latent template (Soha, 2017). The analysis of plastic song in the current study should help clarify when and how the preactive template also begins to contribute to the process of song development, by revealing when normal song syntax and the preference for whistles emerge.

Second, how does song syntax emerge in white-crowned sparrows

with no exposure to syntax information during the sensory phase? Although white-crowned sparrows tutored with individual phrases (including the birds in this study) crystallize fewer phrases than normal, they tend to crystallize songs containing more than one phrase type and to arrange these in species-typical order, beginning with a whistle and ending with other phrase types (Soha and Marler, 2001a; Plamondon et al., 2008). This syntax information must be innately specified. Does this innate specification affect phrase assembly early in song development, or does it act mainly at the end of the sensorimotor phase? To address this, I examined whether the tendency to begin songs with a whistle was present throughout plastic song. I also examined the timing of emergence of crystallized syntax by assessing changes in the diversity of phrase type transitions over development. The results provide a starting point for comparison of syntax development in *Z. l. nuttalli* and in *Z. l. oriantha*, which has been studied in detail by Plamondon et al. (2010).

Finally, how does experience, in the form of early tutor exposure, affect song rehearsal itself? In particular, does syntax information acquired during the sensory stage influence the extent of overproduction and selective attrition? As documented previously (Soha and Marler, 2001a), the single-phrase-tutored birds in this study crystallized songs with fewer phrases than normal. One possibility is that they also memorized and rehearsed fewer phrases than do birds tutored with full, normal white-crowned sparrow songs. Another possibility is that in this subspecies, the number of phrases rehearsed during the sensorimotor phase is itself innately specified and is not influenced by tutor experience. This possibility is supported by the finding that the number of normal tutor songs heard during the sensitive phase did not affect the number of phrases rehearsed in *Z. l. nuttalli* (Nelson et al., 1996b). If the extent of overproduction is innately specified, we would expect birds tutored with single phrases, which lack information about song syntax, to overproduce as much as do birds tutored with normal songs. In this case, the observed crystallization of fewer phrase types would reflect greater repertoire attrition at the end of song development.

2. Methods

2.1. Subjects

Subjects were ten male white-crowned sparrows (*Z. l. nuttalli*) collected as nestlings between four and six days post-hatch from Bodega Head State Park and the Bodega Marine Lab in Sonoma County, CA, in May 1996, and hand-raised in the lab. After fledging, males were identified via laparotomy and placed individually into sound-attenuating chambers where they were maintained on a natural photoperiod for the duration of the experiment. Tutoring began in late May, when birds were between 21 and 26 days old.

2.2. Tutoring and recording

Tutor stimuli were 18 individual phrases extracted from conspecific (*Z. l. nuttalli*) and heterospecific (song sparrow, savannah sparrow, hermit thrush, and American goldfinch) songs (Fig. 1). These phrases included six each of three categories: whistles, buzzes and trills. Within each category, two phrases were conspecific and four were heterospecific. Thus the ratio of conspecific to heterospecific phrases in the tutor set was 1:2 and the ratio of the three phrase types was 1:1:1.

Details of tutoring and recording have been published elsewhere (Soha and Marler, 2001a). Briefly, the individual tutor phrases were presented in bouts of 42 repetitions at 8-second intervals. All phrases in the tutor set were played the same number of times throughout tutoring, with some variation in the sequencing of the bouts. Birds were tutored for four hours daily for 60 days. Five months later, playback of the tutor phrases resumed on a reduced schedule, with the aim of stimulating song production. The same tutor phrases were used at this stage, again in equal proportions.
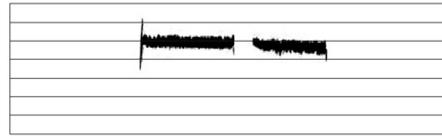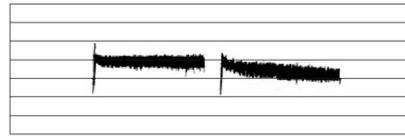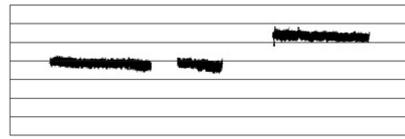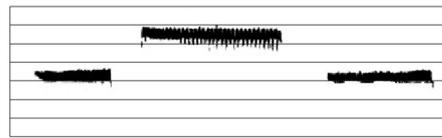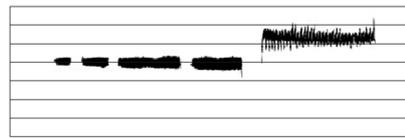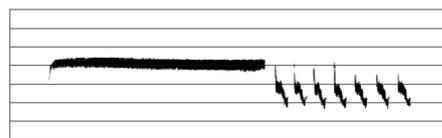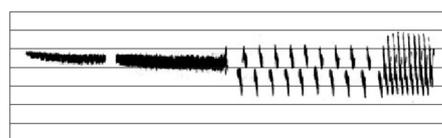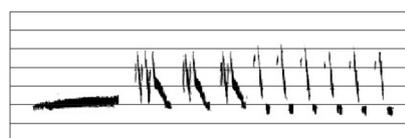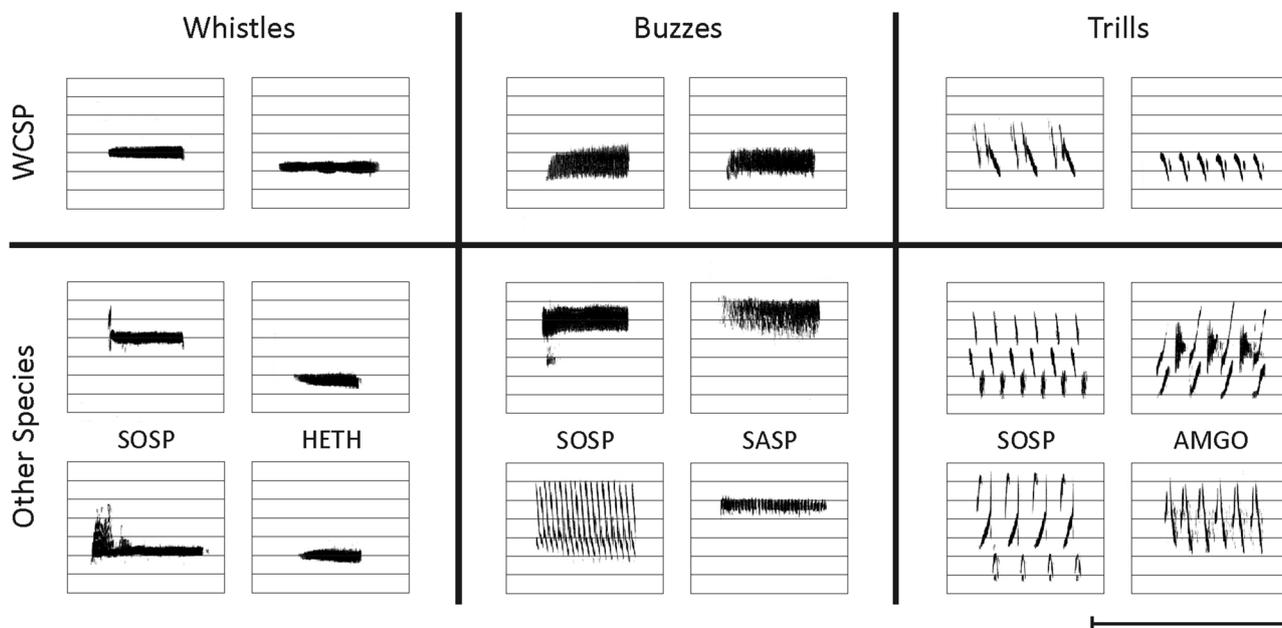
Fig. 1. Single-phrase tutor models (top) and songs learned by the ten male white-crowned sparrows in this study (bottom). All birds were tutored with the 18 phrases shown, including 6 each of whistles, buzzes and trills. Two of each phrase category (top row) were taken from white-crowned sparrow (WCSP) songs and four were taken from songs of othe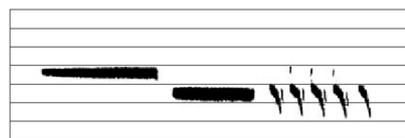r species (SOSP: song sparrow *Melospiza melodia*, HETH: hermit thrush *Catharus guttatus*, SASP: savannah sparrow *Passerculus sandwichensis*, AMGO: American goldfinch *Spinus tristis*). Spectrograms cover 1–8 kHz; time scale bars = 1 s.

The tutored birds were individually recorded for one hour weekly beginning in November, prior to the onset of the sensorimotor phase. Recording continued into March, when crystallized song was recorded from all ten birds. Recording was done between 0730 and 0945.

2.3. Song measurement

All recordings of each bird were assessed and categorized as subsong, plastic song, or crystallized song based on which of these predominated in the recording. These categories can readily be distinguished by ear. Subsong, the earliest vocalization in the sensorimotor phase, consists of highly variable, scratchy sounding elements that contain no imitations. The birds in this study began producing subsong between 189 and 255 days post-hatch. After some weeks of subsong, young sparrows begin to produce plastic song containing elements that resemble adult song notes. These elements, which include phrases the young birds will retain in their own adult song as well as excess (overproduced) phrases, remain variable in structure and sequencing for several more weeks. Gradually this variability decreases until song becomes crystallized, produced consistently across renditions.

Three recordings of the plastic song of each bird were chosen for analysis as follows. The first recording (P1) analyzed for each bird, representing early plastic song, was the earliest recording categorized as plastic song that was not followed by another recording containing predominantly subsong. The second and third recordings (P2 and P3) were chosen from within the subsequent four weeks. Based on whistle phonology, the third recording of each bird represented late plastic song: in contrast to the variable-frequency whistles seen in early plastic song, whistles in these recordings exhibited little frequency modulation. Some variation in phrase types and sequencing was still evident, however, indicating that song was not yet crystallized. P2 was chosen for each bird to be as close as possible to the midpoint between P1 and P3. Exact ages at the P1–P3 recordings differed across birds (Table 1).

At least 46 sequential plastic songs were obtained from each recording. Across birds, an average of 86 P1 songs (range 64–140), 83 P2 songs (51–126), and 78 P3 songs (46–136) were printed as spectrograms for visual inspection. One date of crystallized (C) song from each bird was also chosen and an average of 44 C songs (range 19–61) were printed (Table 1). Spectrograms were printed at a vertical scale of either 30 mm = 8 kHz or 21 mm = 8 kHz and a horizontal scale of 25 mm = 1 s. This resolution was sufficient for phrase identification.

Based on inspection of the printed spectrograms, a catalog of song phrases was assembled for each bird. Phrases were categorized into types based on visual assessment of frequency and, for non-whistle phrases, temporal structure. Each phrase type in C song was identified as either an imitation of a tutor phrase or a non-imitation (either invented or improvised) by the consensus of four judges (Soha and Marler, 2001a). Phrase types in plastic song were identified based on visual comparison both to tutor phrases and to the bird's own

crystallized phrases, by a single judge. The renditions of each phrase type were then counted for each stage (P1, P2, P3 and C). This phrase inventory provided the data for assessment of overproduction and for four calculations of interest: proportions of (1) individual phrases and (2) phrase types that were whistles, and proportions of (3) individual phrases and (4) phrase types that were conspecific rather than heterospecific imitations at each stage of song development.

In addition to the above phrase measurements, measures of syntax were obtained as follows. The number of songs containing more than one phrase, regardless of the number of phrase types, was counted for each stage. Here, songs were defined as groups of phrases separated from other groups by approximately 0.5 s or more of silence. The proportion of multiple-phrase songs that began with a whistle was determined. In addition, all pairwise phrase type transitions occurring within these multiple-phrase songs were tallied.

2.4. Statistical analysis

Spearman's rank correlation tests were used to test for monotonic change across the four recording stages, from early plastic to crystallized song, in each of four repertoire parameters: the proportion of whistles vs. other phrases, the proportion of conspecific vs. heterospecific imitations, the proportion of multiple-phrase songs beginning with a whistle, and the number of phrase types produced. Wilcoxon matched-pairs signed-ranks tests were then used to assess within-bird changes between stages, as follows: for each parameter, P1 and C values were compared first, to test for significant change across the entire developmental range analyzed. If the P1 and C values differed significantly, additional Wilcoxon tests were then used to compare values from successive recording pairs (P1 vs. P2, P2 vs. P3, and P3 vs. C) to investigate in more detail the timing of these changes. For the number of phrase types produced, two tests were done: one including all phrase types, and one including only those phrase types identified as imitations of tutor models. Wilcoxon tests were also used to compare the prevalence of whistles and conspecific phrases at each recording stage to their prevalence in the tutor phrase set, and to compare the proportion of songs beginning with whistles to the proportion of phrases that were whistles at each stage.
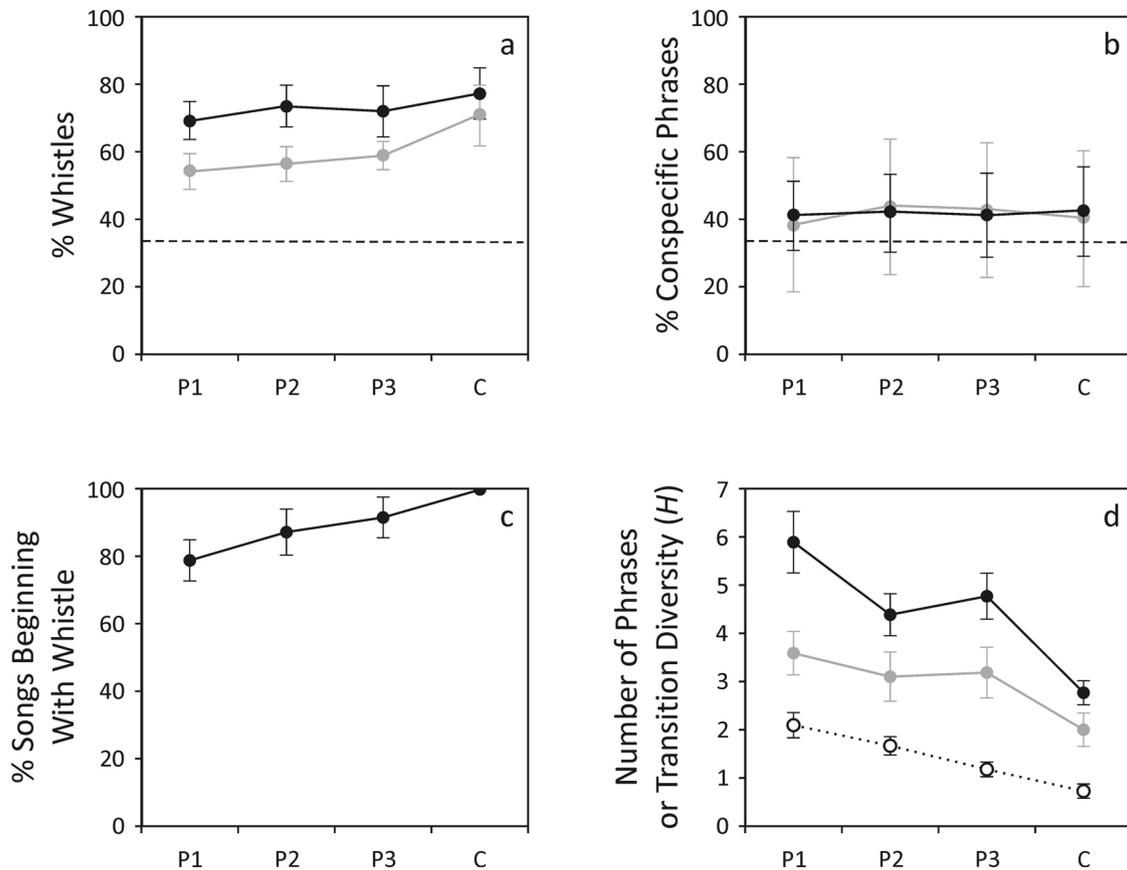
To assess changes over time in phrase sequence diversity, the Shannon diversity index

$$H = -\sum_{j=1}^{s} p_i \ln(p_i)$$

was calculated for the pairwise phrase transitions that were tallied at each stage for each bird. The value of this index depends on both the number of different pairwise transitions (richness) and their relative abundances (evenness). The calculated H values were then compared across stages using a Spearman's rank correlation test and Wilcoxon tests, as described above for the other song parameters.

The extent of phrase overproduction and attrition exhibited by the ten birds tutored with single phrases was compared to that measured in 27 *Z. l. nuttalli* tutored with full, normal white-crowned sparrow song in a previous study (Nelson et al., 1996b). Wilcoxon-Mann-Whitney two-sample tests were used to compare three parameters between the two groups: the number of phrase types recorded in plastic song, the number of phrase types crystallized, and the (within-individual) difference between these. This analysis included imitated phrase types only, as non-imitated phrases were not counted in the Nelson et al. (1996b) study. All statistical tests were done using R software (v. 3.3.3).

Throughout, an alpha level of 0.05 is used. As advocated by Nakagawa (2004), no correction for multiple comparisons was applied. Variables and comparisons were carefully chosen, and the overall pattern of results (Fig. 2) is more informative than the statistical significance of any individual outcome.

Table 1
Ages of birds at each recording, in days post-hatch, and numbers of songs analyzed (in parentheses). P1 represents early plastic song, P2 middle plastic song, P3 late plastic song, and C crystallized song.

	P1	P2	P3	C
b60	284 **(64)**	298 **(51)**	312 **(75)**	319 **(26)**
b62	269 **(75)**	283 **(105)**	290 **(69)**	318 **(59)**
b63	269 **(75)**	276 **(60)**	283 **(136)**	318 **(35)**
b65	263 **(85)**	270 **(126)**	277 **(59)**	319 **(41)**
b66	271 **(80)**	278 **(77)**	285 **(68)**	320 **(19)**
b68	250 **(100)**	256 **(89)**	270 **(87)**	319 **(39)**
b70	263 **(140)**	270 **(56)**	277 **(74)**	319 **(49)**
b73	266 **(86)**	273 **(58)**	280 **(57)**	295 **(57)**
b75	260 **(70)**	267 **(102)**	274 **(110)**	295 **(57)**
b78	259 **(80)**	266 **(101)**	273 **(46)**	315 **(61)**
average	265 **(86)**	274 **(83)**	282 **(78)**	314 **(44)**

Fig. 2. Summary of phrase production in plastic and crystallized song. Graphs show, for each recorded stage: (a) proportion of recorded phrases (black line) and phrase types (grey line) that were whistles, (b) proportion of imitated phrases (black line) and imitated phrase types (grey line) that were conspecific in origin, (c) proportion of multiple-phrase songs that began with a whistle, and (d) pairwise phrase transition diversity *H* (dotted line) and the number of phrase types (black line) and imitated phrase types (grey line) recorded. P1, P2 and P3 represent the three sequential recordings of plastic song for each bird, and C represents the recording of crystallized song. In (a) and (b), horizontal dashed lines indicate the chance expectation. Dates and numbers of songs analyzed are shown in Table 1.

3. Results

3.1. Phrase biases: whistles vs. other phrase types

Fig. 2a shows the proportions of individual phrases and phrase types that were whistles at each recorded stage. No consistent change in the prevalence of whistles across recordings was revealed by Spearman's rank correlation tests either for individual phrases ($\rho = 0.19$, $p = 0.25$) or for phrase types ($\rho = 0.24$, $p = 0.13$). Wilcoxon tests indicated that the proportion of individual phrases that were whistles did not differ significantly between the P1 and C recordings (n = 10 for this and all comparisons, $p = 0.19$), nor did the proportion of phrase types that were whistles ($p = 0.17$). At all four stages, the proportion of whistles recorded was significantly higher than the proportion (6 of 18) presented in the tutor phrase set. This was true both for individual phrases ($p < 0.01$ for all comparisons) and for phrase types ($p < 0.05$ for all comparisons).

3.2. Phrase biases: conspecific vs. heterospecific imitations

The proportions of individual imitated phrases and of imitated phrase types that were conspecific in origin at each stage are shown in Fig. 2b. Spearman's rank correlation tests indicated no increase or decrease across recordings in either of these proportions (conspecific phrases: $\rho = -0.03$, $p = 0.85$; conspecific phrase types: $\rho = 0.01$, $p = 0.96$). Between the P1 and C recordings, Wilcoxon tests indicated no significant difference in the proportion of imitated material that was conspecific in origin, either for phrase types ($p = 0.89$) or for individual phrases ($p = 1.00$). At all stages, none of these proportions differed

from the proportion of conspecific phrases (6 of 18) presented in the tutor phrase set ($p > 0.05$ for all comparisons).

3.3. Syntax development

Fig. 2c shows the proportion of multiple-phrase songs that began with a whistle at each stage. The Spearman's test indicated an increase in this proportion across recordings ($\rho = 0.66$, $p < 0.01$). Crystallized (C) songs all began with whistles; this was higher than the proportion of songs beginning with whistles at P1 (Wilcoxon test: $p < 0.01$). Based on additional Wilcoxon tests, the increase in the proportion of songs beginning with a whistle across two successive recordings was significant between P2 and P3 ($p = 0.02$), but not between P1 and P2 ($p = 0.08$) nor between P3 and C ($p = 0.10$). At all four stages, the proportion of multiple-phrase songs beginning with a whistle was greater than the proportion of whistles (6 of 18) contained in the tutor phrase set ($p < 0.01$ for all comparisons). At stage P1, the proportion of songs beginning with a whistle was no different from the proportion of recorded phrases that were whistles ($p = 0.19$), but at all three subsequent stages, significantly more songs began with whistles than would be expected based solely on the predominance of whistles in the repertoire ($p < 0.05$ for each comparison).

The value of the Shannon diversity index (*H*) for pairwise phrase transitions at each stage is shown in Fig. 2d (dotted line). Pairwise phrase transition diversity decreased monotonically across stages (Spearman's test: $\rho = -0.60$, $p < 0.01$). The difference in *H* between stage P1 and crystallized song was significant (Wilcoxon test: $V = 55$, $p < 0.01$), so further comparisons were done. These indicated that phrase transition diversity was lower at stage P2 than at stage P1

(V = 47, p < 0.05), lower at stage P3 than at stage P2 (V = 47, p < 0.05), and lower in crystallized song than at stage P3 (V = 51, p = 0.01).

3.4. Overproduction

Fig. 2d shows the number of phrase types recorded at each stage (black and grey lines). The total number of phrase types in the repertoire decreased steadily over development (Spearman's rank correlation test, $\rho = -0.56$, p < 0.01), as did the number of imitated phrases ($\rho = -0.37$, p = 0.02). The number of phrase types recorded early in plastic song (P1) was significantly higher than the number included in crystallized (C) song, both for all phrases and for imitated phrases only (Wilcoxon test, p < 0.01 for both). Additional Wilcoxon tests indicated that P1 recordings contained significantly more phrases than did P2 recordings when all phrases were counted (p = 0.02) but not when only imitated phrases were counted (p = 0.17). P2 and P3 did not differ significantly in either total phrase types or imitated phrase types recorded (p = 0.34 for both). P3 recordings contained significantly more phrases than did C song, both for all phrases (p < 0.01) and for imitated phrases only (p = 0.01).

An average of 3.7 (± 1.6 SEM) imitated phrase types were recorded, on any date, in the plastic song of the single-phrase-tutored birds. This did not differ significantly from the 4.4 (± 0.3) imitated phrases recorded in plastic song of *Z. l. nuttalli* tutored with full, normal conspecific song in a previous study (Nelson et al., 1996b; one-tailed Wilcoxon-Mann-Whitney two-sample test, $N_1 = 10$, $N_2 = 27$, W = 108.5, p = 0.18). Despite these similar levels of overproduction, the single-phrase-tutored birds in this study crystallized an average of 2.0 (± 0.3) imitated phrase types, fewer than the 3.3 (± 0.16) crystallized by the full-song-tutored birds (W = 46.5, p < 0.01). This suggests that the single-phrase-tutored birds experienced greater repertoire attrition. Consistent with this, the difference between the number of imitated phrases rehearsed and the number crystallized was greater in the single-phrase-tutored birds (1.7 $\pm$ 0.33) than in the full-song-tutored birds (1.1 $\pm$ 0.57; W = 185.5, p = 0.04).

4. Discussion

Analysis of the plastic song produced by Nuttall's white-crowned sparrows tutored with individual phrases revealed that from the earliest stage recorded, both the proportion of whistles vs. other phrase types and the proportion of conspecific vs. heterospecific phrase imitations were the same as in crystallized song. The proportion of songs beginning with whistles increased and phrase sequence variability decreased during the sensorimotor phase, and these birds overproduced phrases to the same extent previously observed in birds tutored with full, normal song but retained fewer phrase types in their crystallized repertoires. Below, I discuss each of these findings, how they fit together in the overall process of song development, and how they add to our knowledge of subspecies differences in this process.

Results of the present study refine our understanding of how behavioral responses to acoustic cues early in life relate to the process of selective song learning. Upon their first exposure to song as fledglings, young white-crowned sparrows give more 'chirp' vocalizations in response to playback of full conspecific song than to full heterospecific song (Nelson and Marler, 1993). After as few as 10 days of tutoring with multiple songs, young males then preferentially respond to those songs that they eventually learn (Nelson et al., 1997). One might conclude that the predispositions underlying the initial fledgling vocal response are the same as those underlying selective song learning, as Nelson and Marler (1993) suggested. However, results from both playback tests and tutoring experiments using modified songs, including individual phrases, call this conclusion into question. Prior to song memorization, fledgling white-crowned sparrows respond equally to playback of all conspecific phrase types (whistles vs. non-whistles) and respond more to conspecific than heterospecific phrases (Whaling et al., 1997; Soha and Marler, 2001b). The songs that these birds eventually crystallize, in contrast, reflect preferential learning of whistles and no bias towards learning conspecific over heterospecific phrases (Soha and Marler, 2000, 2001a). This suggests that the predispositions underlying the early vocal response differ from those that guide selective song learning.

This difference mirrors the difference between the latent and preactive templates in this species. As described in the Introduction, evidence indicates that in white-crowned sparrows, the preactive template specifies whistles and basic song syntax whereas the latent template is activated by all conspecific phrase types regardless of syntax (Soha, 2017). This conceptual model of preactive and latent templates does not specify the relative timing of action by the two; in particular, preactive templates need not begin to guide song development before latent ones do. If the vocal response of fledgling white-crowned sparrows to all conspecific phrases reflects activation of the latent template at the beginning of the sensory phase, then it appears that in this species the latent template acts earlier in song ontogeny than the preactive template. The current results provide some indication of how and when the preactive template then contributes to song development. From the earliest stage of plastic song analyzed, birds produced more whistles than other phrase types. One possibility is that the preactive template acts during the sensory phase to guide preferential memorization of whistles. Memorization bias cannot necessarily be inferred from rehearsal bias, as evidence from other species indicates that young songbirds can memorize material that they never rehearse (Peters and Nowicki, 2017). Thus it is also possible that the preactive template begins guiding song development only once song rehearsal itself starts. It seems unlikely that the latent template acts alone throughout the sensory phase, however. If this were the case, we would expect the birds in this study to have memorized mostly conspecific phrases. Instead, from the earliest stage of plastic song, these birds produced conspecific and heterospecific phrase imitations in unbiased proportions, that is, in the same proportions as were presented in the tutor set. How then does the preactive template influence song memorization?

Under natural conditions, young birds normally hear full conspecific songs, which meet the specifications of both the latent and the preactive templates. In white-crowned sparrows, these songs begin with a whistle and have a particular basic syntax (as specified by the preactive template), and also contain species-typical non-whistle phrase types (as specified by the latent template). The birds in this study heard a variety of individual song phrases, none of which fully met both sets of specifications. The songs that these birds rehearsed and learned, however, suggest how the templates might normally work together. It appears that in white-crowned sparrows, the latent template initially focuses the young bird's attention on conspecific phrases, as indicated by the preferential response of fledglings to such phrases (Whaling et al., 1997; Soha and Marler, 2001b). In the present study, both conspecific and heterospecific phrases were then memorized and rehearsed, raising the possibility that the commitment to memory of those phrases that activate the latent template depends on whether they *also* meet the specifications of the preactive template. If they do not, a broader range of material (which includes heterospecific phrases) is evidently allowed into memory. Fig. 3 shows a schematic representation of this idea.

Subsequently, the preactive template guides rehearsal of the memorized material, shaping the arrangement of phrases into songs over the course of the sensorimotor phase. As noted above, the preactive template in white-crowned sparrows specifies not only whistles, but also basic syntax: song should begin with a whistle and contain multiple phrases. In this study, the tendency to begin songs with whistles emerged over the course of plastic song. At the earliest stage analyzed, although birds sang more whistles than expected by chance based on whistle prevalence in the tutor set, the proportion of multi-phrase songs beginning with whistles was no higher than expected by chance given the number of whistles produced at that stage. The proportion of songs
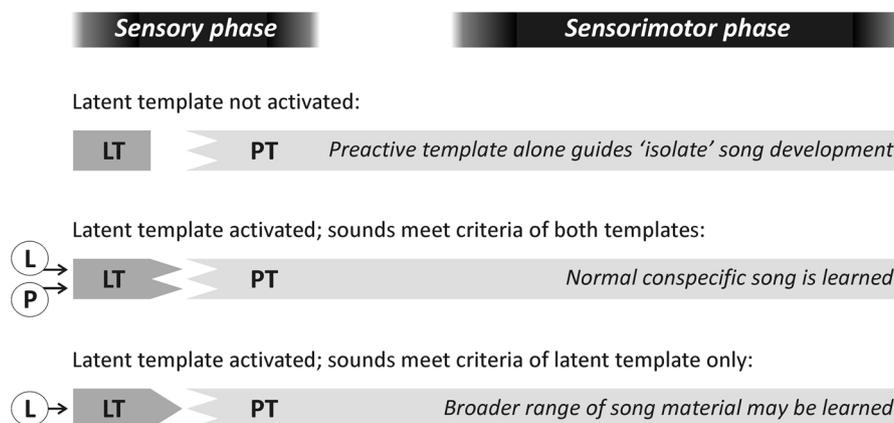
Fig. 3. Schematic summary of how the latent template (LT) and the preactive template (PT) might interact during the sensory phase depending on activation of the latent template, and the results for song learning. Letters in circles represent song (or phrase) models that match the specifications of the latent (L) or preactive (P) templates.

with introductory whistles then increased steadily, with the most significant increase occurring between middle and late plastic song, at approximately 270–285 days post-hatch. This coincided with an increase in the regularity of syntax as indicated by pairwise phrase transition diversity. This diversity decreased significantly from each stage to the next, but the decrease between stages P2 and P3 is particularly informative because during this interval, the number of phrases produced did *not* decrease (see Fig. 2d). The decrease in phrase transition diversity at this time therefore does not merely reflect a decrease in possible pairwise phrase combinations. Earlier in song rehearsal (from P1 to P2), and nearing crystallization (from P3 to C), decreasing phrase repertoire size might partially explain the observed decrease in phrase transition diversity.

Beyond illustrating how the preactive template works throughout the sensorimotor phase, these results provide some basis for comparison of syntax development across species and subspecies. Plamondon et al. (2010) found that in *oriantha*, multiple syntax-related changes occur late in the plastic song of males tutored with individual phrases. These include an increase in phrase bout size (phrases per song), a reduction in phrase pair (transition type) diversity, and a reduction in inter-phrase interval. Phrase pair (transition) diversity is the only one of these particular syntax features analyzed both in that study and in the current one, but generally, both *oriantha* and *nuttalli* males exhibit marked changes in song syntax relatively late in the sensorimotor period. In swamp sparrows, a reduction in the number of phrase types per song occurs similarly late in plastic song, one to two weeks before song crystallization (Marler and Peters, 1982b). Relatively rapid changes in syntax near the end of plastic song appears to be a general feature of song development in new world sparrows, and perhaps more broadly as well. Belgian Waterslager canaries tutored with song models of abnormal syntax (series of individual notes) rehearse these models, but late in plastic song they "reprogram" the learned notes into species-typical syntax (phrases of repeated notes; Gardner et al., 2005). Plamondon et al. (2010) found that *oriantha* males tutored with reverse-order phrase pairs sang predominantly species-atypical (reverse) syntax from the earliest stages of plastic song through cyrstallization, indicating that acquired syntax can override the sequence specifications of the preactive template. Even in these birds, however, the preactive template apparently exerted an effect late in plastic song, when the average number of phrases per song increased to within the species-typical range.

Other aspects of song development have been found to differ even among subspecies of white-crowned sparrow, however. Males of *oriantha* exhibit a shorter sensory phase and longer sensorimotor phase, imitate notes less accurately, and overproduce more (rehearse more phrase types) than do *nuttalli* males (Nelson et al., 1995, 1996a). Interestingly, Nelson et al. (1996b) found that the number of songs heard

during the sensory phase affects the extent of overproduction in *oriantha* but not in *nuttalli*: in the latter, birds tutored with few songs rehearse the same number of phrases as birds tutored with many songs. Here, I found that *nuttalli* males tutored with single phrases rehearsed a similar number of phrase types as did both groups of this subspecies in the Nelson et al. (1996b) study. This indicates that in *nuttalli*, the absence of syntax information in tutor models does not affect the extent of overproduction. Plamondon et al. (2010) found the opposite result in *oriantha*: in that study, birds tutored with full song rehearsed more phrase types than did birds tutored with single phrases. These combined results suggest that the extent of overproduction is innately specified in *nuttalli* but is influenced by phonological and syntactical information obtained during the sensory phase in *oriantha*.

The extent of repertoire attrition in *nuttalli*, however, appears to be affected by syntactical information present in tutor songs. Birds tutored with single phrases exhibited greater repertoire attrition and retained fewer phrase types on average in their crystallized song than did the birds tutored with full songs in the Nelson et al. (1996b) study. In the latter study, similar levels of attrition occurred in birds tutored with many songs or few songs. Together, these results indicate that in *nuttalli*, syntax information, rather than the overall number of phrases heard, affects the extent of repertoire attrition and the resulting number of phrases crystallized in the final song. Syntax information may affect phrase type retention in *oriantha* as well, as single-phrase tutored *oriantha* males also retained fewer phrase types in their crystallized repertoire (Plamondon et al., 2008). However, as noted above, these birds also rehearsed fewer phrase types, and therefore in *oriantha* the extent of repertoire attrition may not depend on external syntax information as it appears to in *nuttalli*.

As the above comparisons illustrate, the details of song development exhibit both similarities and differences in even closely related subspecies. Over a wider taxonomic range of songbirds, considerable variation exists in the process of song ontogeny (Catchpole and Slater, 2008). Comparative study of the auditory template system may provide a useful framework for understanding this variation, in part because the template model can facilitate the integration of neural and ecological levels of analysis (Soha, 2017). The current study illustrates how the detailed analysis of plastic song in specially-tutored birds can further our understanding of the action of auditory templates in song development, and this might prove to be a useful approach in other species as well.

5. Conclusion

Analysis of songs rehearsed by the single-phrase-tutored white-crowned sparrows in this study supports the following conclusions. The selective rehearsal of whistles along with unbiased rehearsal of

conspecific and heterospecific phrases suggest that during the sensory phase in this species, the preactive portion of the template system (which specifies whistles and basic syntax) begins guiding song learning more strongly than the latent template (which is activated by all conspecific phrases). The latent template likely facilitates the memorization of conspecific phrases, but it appears that if these phrases are heard in syntactical context that does not meet the specifications of the preactive template, a broader range of material is also allowed into memory. The syntax specifications of the preactive template further influence song rehearsal throughout the sensorimotor phase, with the largest effect seen shortly (but not immediately) prior to crystallization. Finally, in contrast to the pattern observed in *Z. l. oriantha*, the extent of phrase overproduction appears to be innately specified and the extent of attrition influenced by tutor song syntax information in *Z. l. nuttalli*. These findings futher advance our understanding of the interaction of experience and innate predispositions in song development in white-crowned sparrows.

References

Catchpole, C.K., Slater, P.J.B., 2008. Bird Song: Biological Themes and Variations. Cambridge University Press, Cambridge.

Doupe, A.J., Kuhl, P.K., 1999. Birdsong and human speech: common themes and mechanisms. Annu. Rev. Neurosci. 22, 567–631.

Gardner, T.J., Naef, F., Nottebohm, F., 2005. Freedom and rules: the acquisition and reprogramming of a bird's learned song. Science 308, 1046–1049.

Immelmann, K., 1969. Song development in the zebra finch and other estrildid finches. In: Hinde, R.A. (Ed.), Bird Vocalizations. Cambridge University Press, Cambridge, pp. 61–74.

Kroodsma, D.E., Pickert, R., 1980. Environmentally dependent sensitive periods for avian vocal learning. Nature 288, 477–479.

Marler, P., Marler, P., 1984. Song learning: innate species differences in the learning process. In: Terrace, H.S. (Ed.), The Biology of Learning. Springer-Verlag, Berlin, pp. 289–309.

Marler, P., 1997. Three models of song learning: evidence from behavior. J. Neurobiol. 33, 501–516.

Marler, P., Nelson, D.A., 1993. Action-based learning: a new form of developmental plasticity in bird song. Neth. J. Zool. 43, 91–103.

Marler, P., Peters, S., 1977. Selective vocal learning in a sparrow. Science 198, 519–521.

Marler, P., Peters, S., 1982a. Developmental overproduction and selective attrition: new processes in the epigenesis of birdsong. Dev. Psychobiol. 15, 369–378.

Marler, P., Peters, S., 1982b. Structural changes in song ontogeny in the swamp sparrow *Melospiza georgiana*. Auk 99, 446–458.

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 behavior of sparrows reared in isolation from adult conspecific song. Anim. Behav. 33, 57–71.

Marler, P., Tamura, M., 1964. Culturally transmitted patterns of vocal behavior in sparrows. Science 146, 1483–1486.

Nakagawa, S., 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav. Ecol. 15, 1044–1045.

Nelson, D.A., Marler, P., 1993. Innate recognition of song in white-crowned sparrows: a role in selective vocal learning? Anim. Behav. 46, 806–808.

Nelson, D.A., Marler, P., Palleroni, A., 1995. A comparative approach to vocal learning: intraspecific variation in the learning process. Anim. Behav. 50, 83–97.

Nelson, D.A., Marler, P., Morton, M.L., 1996a. Overproduction in song development: an evolutionary correlate with migration. Anim. Behav. 51, 1127–1140.

Nelson, D.A., Whaling, C., Marler, P., 1996b. The capacity for song memorization varies in populations of the same species. Anim. Behav. 52, 379–387.

Nelson, D.A., Marler, P., Soha, J.A., Fullerton, A.L., 1997. The timing of song memorization differs in males and females: a new assay for avian vocal learning. Anim. Behav. 54, 587–597.

Peters, S., Nowicki, S., 2017. Overproduction and attrition: the fates of songs memorized during song learning in songbirds. Anim. Behav. 124, 255–261.

Plamondon, S.L., Goller, F., Rose, G.J., 2008. Tutor model syntax influences the syntactical and phonological structure of crystallized songs of white-crowned sparrows. Anim. Behav. 76, 1815–1827.

Plamondon, S.L., Rose, G.J., Goller, F., 2010. Roles of syntax information in directing song development in white-crowned sparrows (Zonotrichia leucophrys). J. Comp. Psychol. 124, 117–132.

Slater, P.J.B., Eales, L.A., Clayton, N.S., 1988. Song learning in zebra finches (*Taeniopygia guttata*): progress and prospects. Adv. Study Behav. 18, 1–34.

Soha, J.A., 2017. The auditory template hypothesis: a review and comparative perspective. Anim. Behav. 124, 247–254.

Soha, J.A., Marler, P., 2000. A species-specific acoustic cue for selective song learning in the white-crowned sparrow. Anim. Behav. 60, 297–306.

Soha, J.A., Marler, P., 2001a. Vocal syntax development in the white-crowned sparrow (*Zonotrichia leucophrys*). J. Comp. Psychol. 115, 172–180.

Soha, J.A., Marler, P., 2001b. Cues for early discrimination of conspecific song in the white-crowned sparrow (*Zonotrichia leucophrys*). Ethology 107, 813–826.

Soha, J.A., Lohr, B., Gill, D.E., 2009. Song development in the grasshopper sparrow, *Ammodramus savannarum*. Anim. Behav. 77, 1479–1489.

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.

Whaling, C.S., Solis, M.M., Doupe, A.J., Soha, J.A., Marler, P., 1997. Acoustic and neural bases for innate recognition of song. Proc. Natl. Acad. Sci. U. S. A. 94, 12694–12698.