




The buzz segment of Savannah sparrow song is a population marker

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Abstract

Different components of learned birdsongs change at different rates across generations, and the rate of change may correspond to the information carried by each component. To characterize the role of the buzz segment of Savannah sparrow songs, we examined recordings from southeastern Canada and the northeastern US and fully characterized buzz segments in songs recorded from two populations: one on Kent Island, NB, Canada and another in Williamstown, MA, USA. Buzzes varied geographically: Kent Island buzzes had higher mean frequencies and shorter pulse periods than Williamstown buzzes and the differences between the two populations persisted over time. Population-specific buzz characteristics also appeared to be resistant to change. Variants appeared on Kent Island in the late 1980s and were learned by some younger birds; however, these buzz variants disappeared by 2011. We conducted a playback experiment and found that males from both populations had longer responses to local buzzes. Therefore, buzz structure varies geographically; population characteristics of the buzz persist through time despite the introduction of variant forms; and territorial males discriminate between buzzes from different populations. The learned buzz segment of the song may thus serve as a population marker for Savannah sparrows.

Keywords Song · Cultural evolution · Dialect · Population · Playback · *Passerculus sandwichensis*

Zusammenfassung

Das Buzz-Element des Grasammer-Gesanges ist ein Populationsmarker Verschiedene Komponenten des erlernten Vogelgesanges verändern sich über Generationen hinweg unterschiedlich schnell. Diese Veränderungsrate entspricht möglicherweise dem Informationsgehalt der einzelnen Komponenten. Um die Rolle des sogenannten Buzz-Elements (ein gleichförmiges Schnarren, engl. *buzz*) des Grasammer-Gesanges zu charakterisieren, haben wir Tonaufnahmen aus Südost-Kanada und Nordost-USA untersucht und sämtliche Buzz-Elemente des Gesanges von zwei Populationen ausgewertet: eine Population von Kent Island, New Brunswick, Kanada und eine weitere von Williamstown, Massachusetts, USA. Das Schnarren unterschied sich geographisch: Auf Kent Island wies das Schnarren eine höhere mittlere Frequenz und eine kürzere Pulsdauer auf als in Williamstown. Diese Differenz zwischen den zwei Populationen blieb über die Zeit bestehen. Die Eigenschaften des populationsspezifischen Schnarrens scheinen überdies beständig gegen Veränderung zu sein. Obwohl andere Buzz-Varianten auf Kent Island in den späten 1980ern aufgetaucht sind und von einigen jungen Vögeln erlernt wurden, verschwanden diese wieder bis 2011. Wir führten Playback-Experimente durch und fanden heraus, dass die Männchen beider Populationen länger auf lokales Schnarren reagierten. Zusammenfassend kann man sagen, dass die Buzz-Struktur geographisch variiert, die populationsspezifischen Eigenschaften des Schnarrens trotz der Einführung neuer Varianten über die Zeit bestehen bleiben und territoriale Männchen zwischen dem Schnarren unterschiedlicher Populationen unterscheiden können. Das erlernte Buzz-Element des Gesanges könnte daher als ein Populationsmarker für die Grasammer dienen.

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Introduction

A common characteristic of learned communication systems is the presence of variants that are specific to different populations; when these variants can be mapped to sharp geographic boundaries they are called “dialects” (Marler

and Tamura 1962). For example, the contact calls of yellow-naped amazons (*Amazona auropalliata*) are consistent within geographic areas but vary across regional boundaries (Wright 1996). Within multi-component bird songs, portions of the song, rather than the entire song, may be consistent within populations and thus may serve as markers that encode population identity. Examples include the trill segment of white-crowned sparrow songs (*Zonotrichia leucophrys*, Nelson and Poesel 2007; Nelson 2017), the whistled segment of Grey-cheeked Fulvetta songs (*Alcippe morrisonia*; Shieh et al. 2013), and the whistled portion of Blackcap songs (*Sylvia atricapilla*; Linossier et al. 2015). In contrast, components of learned vocalizations that vary within populations or that change rapidly across generations cannot provide reliable information about population identity. Many bird songs have components with rapid directional or random changes across generations, while at the same time including other components that persist unchanged for decades (e.g. white-crowned sparrows, Chilton and Lein 1996; Nelson et al. 2004; Chestnut-sided Warblers, *Dendroica pennsylvanica*, Byers et al. 2010; Medium Ground Finches, *Geospiza fortis*, Goodale and Podos 2010). To confirm that a component of a complex vocalization is a population marker, it is necessary to show that (1) acoustic features of that marker vary between populations, (2) the characteristic acoustic features are consistent within a population and are stable over multiple generations, and (3) birds respond differently to local and foreign versions of the population marker. Although many studies focus primarily on the first criterion, all three of these criteria must be met for a song feature to be considered a population marker.

Savannah sparrows provide an excellent model for exploring the roles of different song components. Each male sings a single, multi-part song (Wheelwright et al. 2008). Although the components and phrasing of the song differ somewhat across populations, most songs consist of four segments (Fig. 1a): an introduction (an accelerating series of high notes), a middle section (a note complex), a buzz (formed of repeated pulses), and a final segment (which may include a chirp, a trill and/or a distinctive set of notes). The introductory notes appear to be similar across populations and so may denote species, while the middle and final song segments vary both over time and within a population, suggesting that they may encode individual identity (Chew 1981; Bradley 1994; Sung and Handford 2006; Williams et al. 2013). The buzz segment occurs in the songs of male Savannah sparrows recorded throughout North America, and differences in its structure suggest that the buzz may be a population marker. Geographic variation of this widespread Savannah sparrow song component has not previously been quantified, and the function of the buzz has not been tested experimentally.

We collected recordings of Savannah sparrow songs and characterized the acoustic features of buzz segments. We then explored acoustic differences between two populations and asked whether such differences were maintained over multiple decades. To better understand the long-term plasticity of buzz phenotypes, we looked for rare and novel forms of the buzz component within each population and tracked the transmission and fate of such buzz variants over time. Finally, using a playback experiment, we asked whether birds show differential responses to local and foreign versions of buzzes.

Methods

Study sites and Savannah sparrow populations

We conducted detailed studies of Savannah sparrows at two sites: at the Bowdoin Scientific Station on Kent Island, New Brunswick, Canada (44.5818°N, 66.7547°W), and in Williamstown, Massachusetts, USA (42.6645°N, 73.2388°W). The population on Kent Island has been the subject of intensive study since the 1960s, first by Dixon (1978), then from 1987 to 2007 by Wheelwright and colleagues (Wheelwright et al. 1992; Freeman-Gallant et al. 2005), and from 2008–present by Norris and colleagues (Mitchell et al. 2012; Woodworth et al. 2016). For most years in the last three decades, all individuals nesting within a 10-ha study area in the middle of the island (approximately 30–70 pairs each year) have been banded with distinctive color band combinations (Woodworth et al. 2017). Males first breed at the age of 1 year, and the song recordings used for this study span 32 years (1980–2012). Kent Island Savannah sparrows show strong natal and breeding philopatry (Wheelwright and Mauck 1998), and within a given year, birds hatched on the study site represent 40–80% of those recruited to the study site, which is part of a larger population inhabiting Kent Island and two smaller adjacent islands. Because of the systematic banding program, we were able to assign a hatching year for all Kent Island males with songs recorded from 1988 onwards; if a bird was not banded as a nestling or fledgling, it was assumed to be a second-year bird. Paternity for birds hatched from 2001 to 2003 is known from previous work (Freeman-Gallant et al. 2005).

Birds in the population in Williamstown inhabit the edge of an apple orchard and the adjacent hay fields, and males in this population have been followed since 2005. The 10–31 males singing within the 20-ha area have been systematically color-banded and recorded since 2007. The annual return rate of adult males for the Kent Island population is 48% (Wheelwright and Schultz 1994; Woodworth et al. 2017), similar to the return rate of 54% for the Williamstown

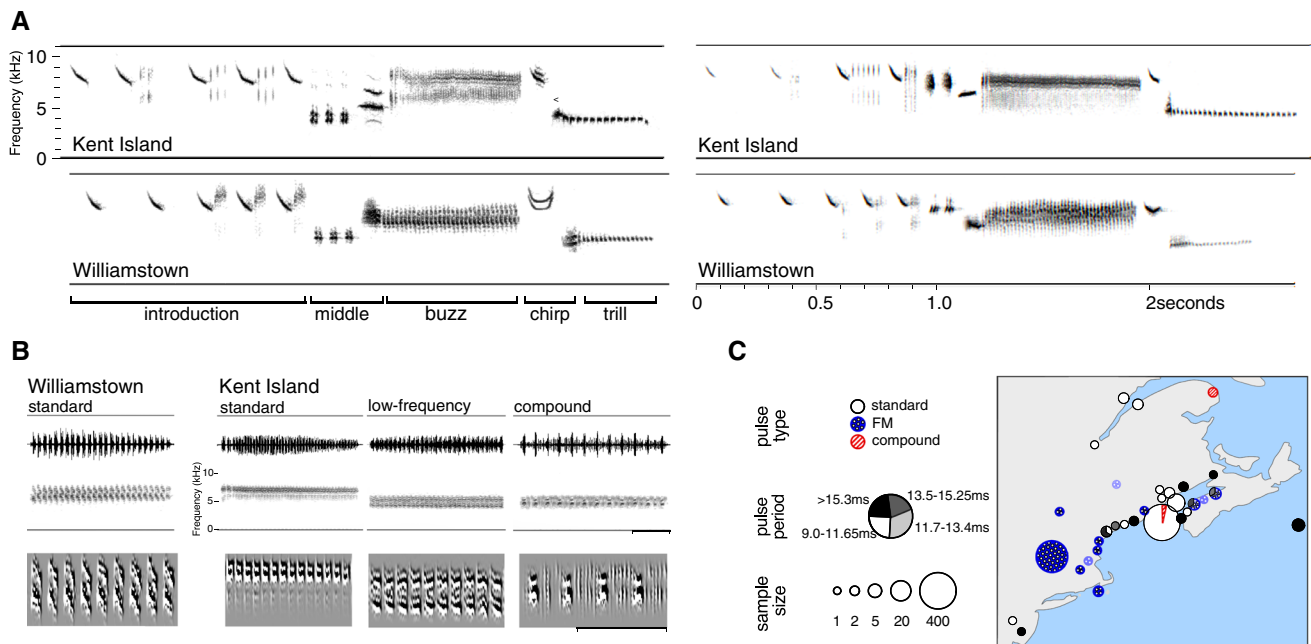


Fig. 1 Variation in the structure of buzzes in Savannah sparrow songs. **a** Two examples of songs, from Kent Island (NB, Canada) and Williamstown (MA, USA), from populations located 600 km apart. Each male sings one song type, and 3–8 song types are present in each location (common song types for each location in 2008–2012 are shown here). All songs consist of the same main segments, including a high-frequency species-typical buzz. **b** Details of representative buzz segments from the two populations. For each buzz type, the top panel shows the waveform and sonogram of the buzz. For better resolution of fine temporal structure, the bottom panel shows a spectral derivative of a short portion of the same buzz. Scale bars correspond to 100 ms. **c** Distribution of buzz characteris-

tics including those drawn from recordings in the Macaulay Library, the Borror Laboratory of Bioacoustics, xeno-canto, and YouTube recordings. The size of the circle denotes the number of recordings from a location, the shade of the fill denotes the pulse period (white: 9–11.65 ms, or within two standard deviations of the mean for Kent Island standard buzzes; dark: > 15.3 ms, or within two standard deviations of the Williamstown mean; other shades: intermediate values). Stippled blue shading denotes pulses with downward frequency modulation, and striped red shading denotes compound pulses. Mixed buzz types in areas with few recordings may represent more than one local population and different time periods, as recordings spanned more than 50 years (color figure online)

population (H.W., unpublished data; nestling return rate for this population is unknown).

Songs and song recordings

We collected recordings of 646 songs sung by a total of 630 male Savannah sparrows. Each male sings only one song type (fewer than 2% sing two different song types; such two-song males account for the difference in number of males and number of recorded songs). A male crystallizes his song early in the first breeding season, and only very rarely changes the song thereafter (of the 131 birds that bred for more than 1 year between 2003 and 2012, one changed his song type, one added a trill in the second year, and two sang shortened and attenuated buzzes during their final breeding season but did not change their song types). Thus, a recording of a male at any point during or after the first breeding season represents the song used throughout his lifespan. Recordings spanning a period of more than 30 years from Kent Island were used in our analysis ($n = 457$ males and 471 songs; for details of recordings and methods, which changed

as technology improved across decades, see Williams et al. 2013). Breeding males in Williamstown ($n = 94$ males, 96 songs) were recorded from 2007 to 2012 with a Marantz digital recorder (PMD670 or PMD660) and a Sennheiser ME-66 microphone. Savannah sparrow males returning to breed on Kent Island have an average lifespan of 1.5 years, but can live up to 8 years (Wheelwright and Rising 2008). To consider changes over time, we used (1) hatching year cohorts (i.e. all males hatched in a single year) to track the origin of changes in buzz forms, and (2) singing year cohorts (i.e. all males present during a breeding season, regardless of age), to determine the forms present in a given year and when they were lost. For all years analyzed in detail, recordings were obtained for 75–100% of the males present on the study areas. Examples of song recordings are available at <https://doi.org/10.7479/8zrt-j1tg>.

To compare properties of the buzz segments of songs from the two main study areas to those from a broader geographical area, we measured buzz characteristics from 79 Savannah sparrow songs recorded in northeastern North America from a variety of sources: (1) our own recordings

made on Grand Manan Island, New Brunswick, and other islands near Kent Island (2011–2, $n = 16$), (2) Clara Dixon's 1980–1982 recordings from Grand Manan Island and other locations along the coast of the Bay of Fundy in Nova Scotia and New Brunswick ($n = 31$), (3) several locations from recordings archived in the Macaulay Library of Natural Sounds (1951–1982; $n = 10$), (4) several locations from recordings archived in the Borror Laboratory of Bioacoustics (1955–1960; $n = 6$), (5) several locations from recordings archived at xeno-canto.org ($n = 9$, 1998–2016), and (6) several locations from recordings derived from other internet sources, primarily YouTube ($n = 7$, 2009–2015). Recordings from the internet were screened and used only if the signal/noise ratio was appropriate for analysis. Supplementary Table 1 lists locations, dates, and source numbers for the recordings derived from libraries and other outside sources.

Song analysis

We characterized each recorded buzz by measuring fine structural details of acoustic characteristics. Before measurements were made, all analog recordings were digitized using SoundEdit Pro (Macromedia, San Francisco, CA; see Wheelwright et al. 2008). The song with the best signal-to-noise ratio from each recording was used for analysis. Where appropriate, the extraneous noise that fell in frequency ranges that were entirely below or above that of the buzz segment were filtered out using high- and low-pass filters implemented in SoundEdit 16 or Audacity (audacityteam.org). SoundAnalysis Pro (Tchernichovski et al. 2000; <http://soundanalysispro.com>) was used to measure the overall duration (ms) and mean frequency (kHz) of each buzz. The number of pulses (repeated sound units) in each buzz was counted, and the buzz duration was divided by the number of pulses to obtain the pulse period. The pulse type was scored as compound (two-parted, with a harmonic section plus a click section), frequency-modulated (descending pitch during each pulse, as in Williamstown buzzes), or standard (a single, unmodulated pulse rich in harmonics; see Fig. 1b for examples).

To determine whether buzzes with distinctive pulse types (see Fig. 1) were transmitted from father to son, we visually compared spectrograms of songs produced by males and their sons. If the buzzes were of different types, we inferred that learning from the father had not occurred (see Wheelwright et al. 2008). Spectrograms were produced using SoundEdit 16 (Macromedia) or Audacity (<https://www.audacityteam.org>), using 512 point Hamming windows.

Playback experiment

We conducted a playback experiment using recordings of buzzes from the Kent Island and Williamstown study

populations to evaluate how the birds in these two populations responded to local and foreign buzzes. In pilot studies, males responded strongly to stimuli consisting solely of local buzzes (H.W., unpublished data), so we presented isolated buzzes outside the context of song; this allowed us to avoid the possibility that birds' responses might be affected by some other aspect of the song. Sound stimuli were prepared using SoundEdit Pro from songs recorded on the Kent Island and Williamstown study sites, and consisted of a 0.4–1.0 s buzz section of a song embedded in a 12-s silent period, yielding, when repeated, a rate of five stimuli per minute. A total of 15 stimulus sets were prepared from recordings of 30 birds no longer present on either study site, with each set consisting of one Williamstown buzz and one Kent Island buzz. For each stimulus set, we normalized the stimuli to the same maximum amplitude and we standardized the duration within 5 ms (we accomplished this by deleting pulses from the central portion of the longer of the two stimuli using SoundEdit Pro). A “primer” stimulus, used to attract birds to the playback site, consisted of a local song exemplar (either Kent Island or Williamstown) in which the buzz segment was replaced by silence. All stimuli were derived from files recorded in wav format, saved in the same format, stored on an iPod and presented through a Realistic Mini-mus 0.6 speaker placed 2 m from a song perch the subject had recently used. Two observers (CR and HW) crouched 10 m from the speaker; birds readily approached the speaker despite the presence of the observers. Stimulus intensity was measured as 70 dB at 2 m from the speaker using a Bruel and Kjaer sound meter (fast response and A weighting).

Playback sessions began between 0730 and 1130 h in early July, when paired males readily responded. No neighboring males served as subjects on the same day. The speaker was placed near the center of the defined territory of the color-banded male subject. In our study areas, territories are relatively small (~40 m in diameter), and playbacks were performed away from boundaries to avoid triggering responses by neighboring birds. In preliminary studies, breeding status (date, and whether the male was feeding young) did not affect the responses of mated males at either site (H. Williams, unpublished data; $P > 0.9$) and so these variables were not included in the analysis.

Each session began with the primer stimulus, which was repeated until the target male approached within 2 m and heard at least ten songs (we aborted the session if no male approached the speaker within 3 min). Responses usually continued after the playback of the primer stimulus had ceased. Response duration was timed and ended when the subject performed any one of the following behaviors: (1) moving more than 20 m away from the speaker, (2) feeding, (3) preening, or (4) performing a loud song. Loud song occurred when (a) the singer's beak was opened while singing, (b) the head tilted back from the resting position during

singing behavior, and (c) sound amplitude was that of normal territorial song. In contrast to soft song, which is associated with aggression in Savannah sparrows (Moran et al. 2018) as well as song sparrows (*Melospiza melodia*, Searcy et al. 2006), loud song most often occurred before the birds responded (birds that sang loud song during the playback period approached the speaker with longer latencies in pilot studies than those that did not sing loud songs, $F_{(1,44)} = 6.75$, $P = 0.013$). When the bird had ceased responding to the primer stimulus, a 2-min pre-playback silent interval began.

After the primer stimulus, the end of the response to that stimulus, and the 2-min silent period, the playback session continued with presentation of two local and two foreign buzz types in alternation (see Fig. 2). The first buzz was played for 2 min (10 buzzes). After the bird stopped responding (as defined above), a 2-min silent period began, followed by the second buzz type for 2 min, and then again 2 min of silence after the bird stopped responding (as defined above). This block of two trials was then repeated so that each buzz type was presented twice, in alternation. By waiting for 2 min after a bird stopped responding to the primer or to the previous buzz type, we attempted to standardize the subject's response state at the beginning of all stimulus presentations.

Four males' initial response time to the primer was so long (extending more than 30 min after the primer stopped playing) that the buzz type presentations were reduced to 1 min per trial to avoid exhausting the playback subjects. To account for differences in primer playback duration and stimulus presentation duration, both of these variables were included in statistical models assessing responses to playback stimuli; see below.

The stimulus set used was changed for each playback trial, and the order of initial stimulus was alternated across completed playback trials. A total of 58 playback sessions were initiated, but because we required that males respond, that they be in sight on their territories for the entire sequence, and also that no intrusions occur, only 18 sessions (Kent Island = 11, Williamstown = 7) were used for analysis. These sessions remained counterbalanced for stimulus order presentation; Kent Island birds heard the

Kent Island buzz first in five sessions, while Williamstown birds heard the Kent Island buzz first in four sessions, and no stimulus set was used more than once in either location.

In related studies, we compared response duration to other response measures (see Supplementary Table 2). Although response duration is not a direct measure of aggression, we found that long responses were strongly correlated with close approaches to the speaker and the number of aggressive wing flutters (see Wheelwright and Rising 2008). However, in the pilot studies birds gave wing flutters in only 32% of trials, while the closest approach measure was often constrained by the small territory sizes in our populations. Thus response duration proved to be the most useful measure for comparing birds with different behavioral profiles and was our primary metric.

Statistical analyses

We tested hypotheses about population differences in and changes over time of several acoustic measures of buzzes (duration, mean frequency, pulse period) using traditional statistical methods. The distribution of parametric buzz measures was tested for fit to a normal distribution. The distribution of these measures did fit a normal distribution within each of the four buzz types (see Results), so we used t tests for comparisons of buzz types. However, the overall distribution of these measures in the Kent Island population, which included three buzz types in some years, was not normal when multiple buzz types were present, so a Mann–Whitney U test was used to compare buzzes at the population level. We used regressions to assess the stability of buzz parameters recorded from individual birds across successive years. We report uncorrected P values and set the level of statistical significance at $P = 0.05/3 = 0.016$ (Bonferroni correction for comparing three acoustic variables).

For the analysis of playback data, we used generalized mixed-effects linear models implemented within the lme4 package in R (Bates et al. 2015, www.R-project.org) and a model selection approach. We focused on response duration (defined as time from the last stimulus in a playback period until the bird ceased responding; see Fig. 2). We tested multiple models that included the variables of stimulus type and population. All models also included the following variables as random effects: bird identity, trial order, primer playback duration, and stimulus trial duration. Models were ranked using AIC values. To test the effect of the interaction between stimulus source and location of the playback, models with the lowest AIC values were compared to a null model that lacked the interaction, and the significance of the variable that was not included in the null model was evaluated with the Chi-squared statistic (see Winter 2013).

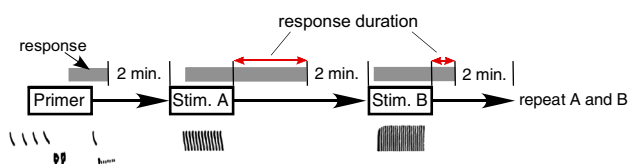


Fig. 2 Schematic of the playback experiment used to test the responses of Savannah sparrows to local and foreign buzzes. The silent interval between playback stimuli consisted of the response duration after the playback stopped plus an additional 2 min. Response duration was measured as the time that a male continued responding after the last stimulus in a playback period was played (color figure online)

Results

Buzz types

A total of four buzz types were sung by Savannah sparrows on Kent Island and in Williamstown (Fig. 1a, b; Table 1). All Williamstown males sang the same buzz type ($n=93$; 2008–2012). Kent Island males sang three different buzz types; more than 90% of the 451 Kent Island males recorded between 1980 and 2012 sang the “standard” Kent Island buzz type. The two populations’ standard buzz types can easily be discriminated by ear; the Williamstown buzzes have a lower pitch and seem to “rattle” in comparison to the Kent Island standard buzzes.

Two variant forms of the Kent Island buzz (Fig. 1b, Table 1) were sung by 38 males (8.4%) on Kent Island (four birds, 0.9%, sang songs without buzzes). Buzz variants were absent from recordings made in 1980 and 1982, first recorded in 1988, and last recorded (from one 7-year-old male) in 2010. The first variant, which we call the “low-frequency variant”, was sung by 19 birds and had pulses that were structurally similar to those of the standard buzz but of a significantly lower mean frequency ($t=26.8$, $df=425$, $P<0.001$). The second variant, which we call the “compound buzz”, was also sung by 19 birds; it had a mean frequency similar to that of the low-frequency buzz ($t=0.8$, $df=36$, $P=0.43$) and significantly lower than that of the standard Kent Island buzz ($t=28.7$, $df=425$, $P<0.001$). Instead of single uniform pulses, the compound buzz consisted of a repeating subunit formed by one pulse followed by a closely spaced series of clicks. The subunits, or pulses, that made up the

compound buzzes were significantly longer than those of other Kent Island buzz types ($t=63.8$, $df=444$, $P<0.001$). The differences between the two Kent Island variant buzz types and the standard buzz can be distinguished by ear (examples of all buzz types are available at <https://doi.org/10.7479/8zrt-j1tg>).

Population differences in buzz characteristics

We compared the acoustic parameters of Kent Island and Williamstown buzzes for the 5-year period from 2008 through 2012, when simultaneous comprehensive recordings were available from both study sites ($n=132$ for Kent Island, $n=92$ for Williamstown). The pulse period was significantly shorter for Kent Island buzzes (Fig. 3a; $U=12.73$, $P<0.001$), and the mean frequency was lower for Williamstown buzzes (Fig. 3b; $U=7.14$, $P<0.001$). Buzz duration did not differ significantly between the two populations (Fig. 3c; $t=1.3$, $df=222$, $P=0.20$).

The pulse type and pulse period of Savannah sparrow buzzes recorded in several locations across four decades in New England and southeastern Canada are summarized in Fig. 1c. Some regional structure of buzz segment characteristics appears to exist—for example, buzzes recorded near the mouth of the Bay of Fundy tended to be similar, as did those recorded in southern New England—and more patterns might emerge with fine-scale, systematic mapping. Because relatively few exemplars (usually one or two) are available from each of the additional locations shown in Fig. 1c, we did not perform systematic analyses of the acoustic characteristics for each of these locations.

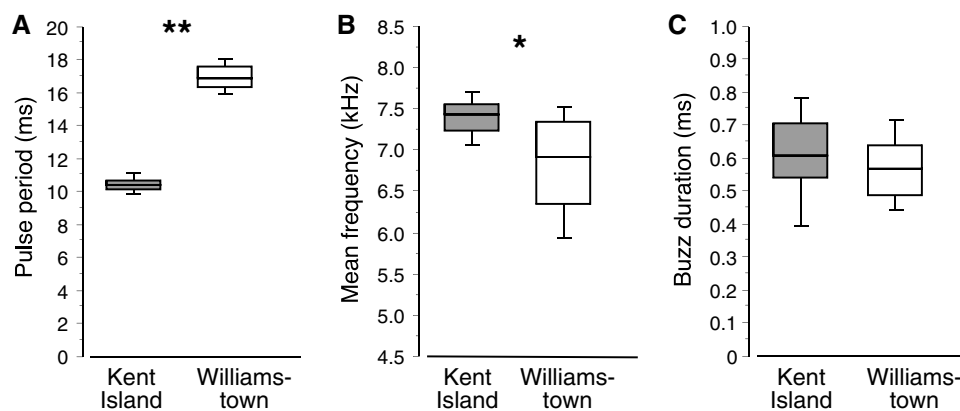


Fig. 3 Acoustic features of buzzes from Kent Island and Williamstown Savannah sparrows, 2008–2012. The central line of the box plot denotes the median value, the upper and lower bounds of the box the 25th and 75th percentiles of the distribution, and the error bars the 10th and 90th percentiles for **a** pulse period, **b** mean frequency, and **c** duration of buzzes. Asterisks denote significant differences between

populations ($*P<0.0001$; $**P<0.00001$). During the 5-year period summarized in this figure, only three of the 132 males recorded on Kent Island sang buzzes with low-frequency pulses (all are included in the figure and analyses); hence the mean frequency for all Kent Island buzzes is similar to that of the standard buzz (color figure online)

Buzz characteristics are consistent within populations and stable over time

We asked whether the acoustic features that best distinguished the Kent Island and Williamstown buzzes, mean frequency and pulse period, were stable and consistent over time for each population (Fig. 4). Although pulse period decreased slightly in both the Kent Island and the Williamstown populations, the R^2 value was low and there was no evidence that the slope of the relationship was different from zero ($R^2=0.01$, $n=446$, $P=0.08$ for Kent Island; $R^2=0.03$, $n=92$, $P=0.18$ for Williamstown). The mean frequency of the Williamstown and Kent Island buzzes did not vary across years (Pearson $R^2=0.00002$, $n=92$, $P=0.97$ for Williamstown; $R^2=0.0004$, $n=446$, $P=0.67$ for Kent Island). When variant buzzes were excluded, the mean frequency of standard Kent Island buzzes was consistent across decades ($R^2=0.006$, $n=408$, $P=0.12$). Thus mean buzz frequency was consistent over time regardless of whether variant forms were included in or excluded from the analysis.

The pulse type, buzz duration and the mean frequency of an individual male’s buzz were also consistent over time. Between 2007 and 2012 on Kent Island, 48 individual males with standard buzzes were recorded in both their first and second breeding seasons, and the mean frequency and duration of five buzzes from each year from each bird were averaged. No males changed their pulse type, and individual males’ buzz characteristics were strongly correlated across years (for duration, $R^2=0.58$, $n=48$, $P<0.0001$; for mean frequency $R^2=0.45$, $n=47$, $P<0.001$).

Males discriminate buzzes from different populations

Male response duration was significantly longer to buzz stimuli derived from the local population (Fig. 5; $\chi^2=21.29$,



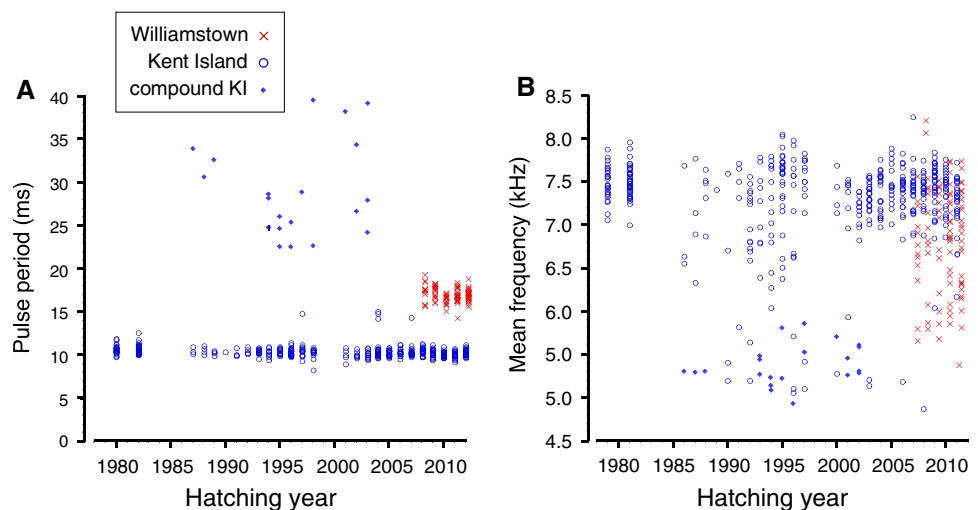
Fig. 5 Male Savannah sparrows responded for longer to local buzz types. Both Williamstown and Kent Island males had longer responses to buzzes from their own population. Response duration was measured as the time from the end of the stimulus to the end of the response (see “Methods” and Fig. 2)

$df=3$, $P<0.0001$). We asked whether longer responses to the local buzz might have been in part due to population differences in male responsiveness or in stimulus effectiveness in evoking responses. Kent Island and Williamstown buzzes were equally effective at eliciting responses ($\chi^2=1.43$, $df=1$, $P=0.23$). Although birds on Kent Island appeared to respond for longer to all stimuli than did Williamstown birds, that difference was not significant ($\chi^2=2.32$, $df=1$, $P=0.13$). Thus the stimulus source and the population of the responder did not affect response duration, but local buzzes elicited longer responses than did foreign buzzes from males of both populations.

Buzz variants and learning

Stable population differences in the form of the buzz segment could be maintained by cultural transmission via social learning or by genetic mechanisms. To assess the possibility

Fig. 4 Savannah sparrow buzz characteristics over time. **a** Mean pulse period as a function of hatching year. **b** Mean frequency as a function of hatching year. Values for Williamstown buzzes are shown with red “x” symbols, blue open circles denote standard and low-frequency Kent Island buzzes, and blue filled diamonds designate the Kent Island compound pulse buzz variant (color figure online)



that genetic background contributes to the buzz form that a male sings, we looked at the family histories of males singing the uncommon buzz variants on Kent Island. We first asked whether young males that sang buzz variants had fathers that sang variants (Fig. 6a). Nine males singing buzz variants had been banded as nestlings and had fathers with recorded songs, but only one of those fathers also sang a buzz variant (in that case both the father and the son sang compound buzzes). We next asked whether fathers that sang buzz variants passed their buzzes on to their sons. Only one of ten returning sons of fathers that sang buzz variants also sang a buzz variant. Since extra-pair paternity occurs in Savannah sparrows, we also considered males that had known paternity and had a genetic or social father that sang a variant buzz or sang variant buzzes themselves. Of those nine males, only three sang buzzes that matched those of their genetic fathers.

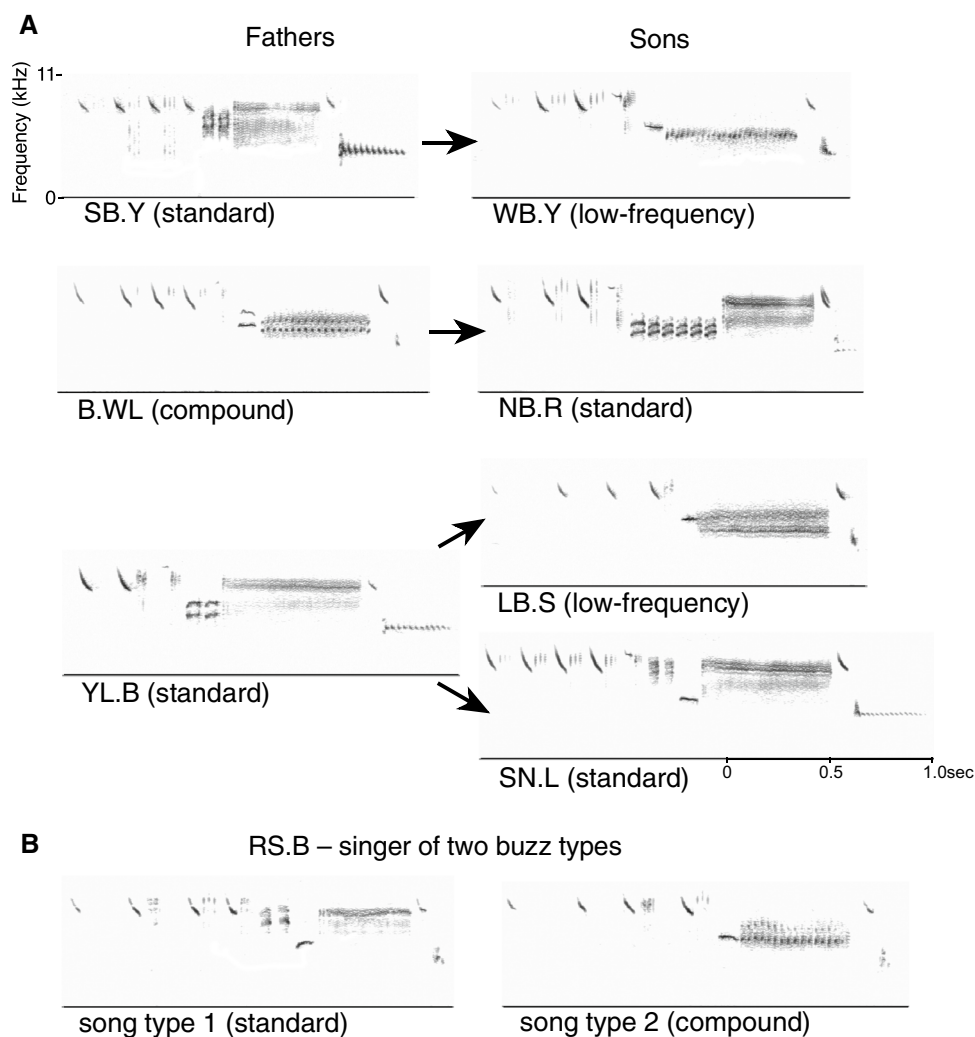
We also considered the seven Savannah sparrow males that sang two songs instead of the usual one (Fig. 6b). Four of those birds sang songs that had identical buzzes, but three

males sang one song with a standard buzz and one with a variant (two of these sang low-frequency buzzes and one a compound buzz), showing that individual males are not constrained in their choice of buzz type during song learning.

Discussion

Our results indicate that the buzz segment of the song characterizes Savannah sparrow populations: it varies geographically and is stable over time, and the local form is discriminated by males. Williamstown buzzes are lower in mean frequency and are made up of longer pulses than Kent Island buzzes. These characteristics were consistent over years (Williamstown) and decades (Kent Island); when unusual buzz variants appeared, they did not persist in the population. Males responded for longer to their own population's buzz type. These results strongly suggest that the buzz segment of Savannah sparrow song carries information about the population of the singer. The features of a vocal dialect

Fig. 6 Evidence that buzzes are not genetically inherited. **a** Sons' buzz types did not necessarily match those of their fathers. SB.Y was the social father of WB.Y (no paternity testing was done for this bird); B.WL was both the genetic and social father of NB.R; LB.S and SN.L hatched in the same nest and had the same social father (YL.B) and genetic father (EPP: Y.RL), but sang different buzz types as adults (compare the frequencies of the buzzes in reference to the note immediately following each buzz). **b** An example of an individual male that sang both standard and variant buzzes



include geographic differences and stability; an additional criterion is a clear boundary between different acoustic forms (Marler and Tamura 1962), although this specification has not always been met in studies reporting song dialects (vide Mundinger 1975). Clear boundaries between Savannah sparrow populations singing different buzz forms may exist. However, our data on buzz forms across the distance between Williamstown and Kent Island does not have a sufficient sampling density to define such boundaries, and hence we use the more conservative term “population marker”.

Relatively few studies have directly asked whether components of multi-part learned vocalizations are sufficient to mark regional or population identity, as our evidence suggests the buzz segment does for Savannah sparrow songs. The trill segment of the White-crowned sparrow song also appears to play a role as a regional marker; playback stimuli that replace the local trill with a foreign trill elicit weaker responses (Nelson and Soha 2004), and the whistled phrase of the song of the Gray-cheeked Fulvetta plays a similar role (Shieh et al. 2013). The trill syllables of the White-crowned sparrow song are more stable over time than are “complex syllables” from other portions of the song (Nelson et al. 2004). This long-term stability of both the White-crowned sparrow trill and the Savannah sparrow buzz is consistent with a role for such song components as population markers.

Our playback experiment demonstrates that the sparrows perceive and discriminate population differences in the buzz segment. Both Williamstown and Kent Island males responded for longer to buzzes from their own population. A finding of stronger responses to the local song is consistent with many other studies (reviewed in Nelson 1998). A number of reasons for a stronger response to an incursion by a male singing the local song type have been advanced, including the possibility that matching the local form might occur because it is a conventional signal of aggressive intent (Vehrencamp 2001) or because a matching signal allows an eavesdropper to readily make a direct comparison between two singers (Mennill et al. 2002; Logue and Forstmeier 2008).

Socially learned behaviors can change rapidly due to copying errors, improvisation, and immigration by individuals that use different forms of the behavior (Boyd and Richerson 1985). Although such phenomena allow cultural evolution in bird songs to occur over relatively short time spans, other song types or song segments may evolve more slowly (Zimmerman et al. 2016; Nelson et al. 2004; Byers et al. 2010). In sharp contrast with rapid change in other song segments over the same time span, buzz characteristics were stable over more than three decades on Kent Island (Williams et al. 2013), which is consistent for a role in carrying information about population rather than individual identity.

The stability of the buzz segment in the Kent Island population was challenged by two unusual low-frequency buzz

variants that appeared during the mid-1980s. The source of these variants is unknown, but it is interesting to note that one of the variants also appears in a single Macaulay Library recording from the Gaspé Peninsula (Fig. 1; approximately 400 km north of Kent Island); perhaps immigration by one or more birds from that area introduced the variant buzz form. As Savannah sparrows do not alter the songs they sing after their first breeding season (Wheelwright et al. 2008), a new variant persists as long as its singer is alive, and during that time it may also be copied by younger birds. In this scenario, an initial increase in the proportion of birds singing a variant, as occurred on Kent Island in the early 1990s, may have been caused by random events.

Differences in the structure of the two populations might have had an impact on variation in buzzes. Kent Island, though small in area, is rich in suitable habitat and has a population of hundreds of males with relatively small territories. In contrast, suitable habitat in Williamstown is patchy and scattered, and birds occupy larger territories. Birds from the Kent Island population are thus likely to hear more adult males (and more different songs) during development, which might favor variability in buzz type. Despite these differences in population structure, buzz types were stable over time in both locations. Only two Kent Island males hatched after 2003 sang a buzz variant. The last of these failed to return after 2010. All of the birds recorded on the study site in subsequent years sang the standard Kent Island buzz. Thus, despite the perturbation provided by the introduction of variant forms, the structure of Kent Island buzzes in 2012 was identical to that in 1980.

Although learning is critical in the development of passerine song (Marler 1997), one possible explanation of buzz structure stability over time within a population is that some vocal characteristics, such as frequency, may have a genetic basis, perhaps by way of indirect mechanisms such as heritable body size (Forstmeier et al. 2009) or sensory biases (Mundinger 1995). However, our results confirm and extend those of Wheelwright et al. (2008) in finding that Savannah sparrow buzz characteristics are learned from adult males, which may include genetic fathers, social fathers, and neighbors. Singers of standard Kent Island buzzes fathered singers of variant buzzes and vice versa, brothers from the same nest and with the same genetic and social parents sang different buzz types, and among the small number of males that sang two song types, some sang two different buzz types. These observations make genetic transmission of buzz types an unlikely explanation for the maintenance of a local form of the buzz song segment.

How and why vocally learned population (or dialect) markers are maintained is not well understood. Hypotheses about the bases of stable regional differences in learned vocalizations include that they (1) are an epiphenomenon of vocal learning (Baker and Cunningham 1985; Planqué

et al. 2014), (2) allow for local adaptation through female choice of familiar-sounding males (Baker and Mewaldt 1978), (3) arise secondarily from population differences in morphological or physiological characteristics that influence sound production (Podos and Nowicki 2004; Prather et al. 2012), (4) result from ecological constraints favoring certain sound characteristics (Morton 1975; Slabbekoorn and Smith 2002), (5) represent a social adaptation with sexual selection benefits (Payne 1981; Nelson et al. 2001), or (6) result from conformist learning (Mesoudi 2016), perhaps via mechanisms such as small learning biases in the transmission of song (Kirby et al. 2007) or behavioral enforcement of conformity (Lachlan et al. 2004). The long-term Savannah sparrow study on Kent Island, coupled with the natural experiment of an introduction and subsequent loss of buzz variants, offers the potential to better understand how stable population differences in learned vocalizations are maintained.

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Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Procedures were carefully considered with respect to their effects on the birds, and were reviewed and approved by the Bowdoin College Research Oversight Committee (2009-18 r2011), the Williams College IACUC (WH-D-09), and the University of Guelph Animal Care Committee (08R601). Studies were carried out as specified by the US Fish and Wildlife Service (banding permits 02109 and 22516) and the Canadian Wildlife Service (banding permit 10789D). All experiments complied with the current laws of the US and Canada.

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