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Time alters urban singing strategies in a North American songbird

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Abstract

Local habitats shape animal vocalizations through selection to improve transmission of signals to receivers. This process can be variable, however, when landscapes are changing continuously due to urbanization or other factors. Studies have shown that some birds alter the frequency, amplitude and structure of their songs in urban habitats. Because songbirds learn their songs from other individuals, this cultural transmission can result in substantial change over time. Urban bird populations may therefore show rapid shifts in song form and variation may result from the combined or interacting effects of time and anthropogenic habitat change. In this study, we used historic and recent song recordings to investigate changes in spotted towhee (*Pipilo maculatus*) songs over 45 years across an urban-to-rural gradient in northern California. We found that pre-trill and trilled song parts covaried differently with urban development at the two time-points, with more change occurring in trill phrases than pre-trills. Additionally, we found that birds in 1970 adjusted trills in urban areas by raising maximum frequencies and broadening bandwidths, while birds in 2015 narrowed song bandwidths by decreasing maximum frequencies in more urban areas. These results did not fit our prediction that urbanization would have a consistent effect on song at two time-points. We suggest that habitat, cultural evolution and cultural drift can act on song elements in complicated ways that vary over time.

Key words: cultural evolution, bird song, *Pipilo maculatus*, urbanization, animal communication

Introduction

Animal signals vary in relation to local habitats and this variation can improve transmission to a receiver (Morton 1975; Ey and Fischer 2009). For animals that learn their communication behaviors, signals have the potential to adapt to environments relatively rapidly through cultural evolution across time and space (Luther and Derryberry 2012). While changes in animal signaling systems occur regularly over evolutionary time, human-altered environments also have been shown to change

those same signals on much faster time scales (Smith and Harper 2003; Warren et al. 2006; Derryberry et al. 2020). In a changing world, an organism's ability to tolerate anthropogenic environmental changes may depend on its ability to adopt new behaviors or modify existing ones in altered environments (Luther and Baptista 2010; McDonnell and Hahs 2015).

Bird song provides an excellent system within which to study changes in learned communication signals. Due to the rapid increase in urban noise and changes to landscapes, bird song has become popular for studying the effects of human-

altered environments on animal signaling traits (Shannon et al. 2016). Some birds learn their songs (Beecher and Brenowitz 2005) and use these signals in mate attraction and intrasexual aggression (Catchpole and Slater 2003). Increasingly, studies have demonstrated that songbirds alter their songs in urban areas by modifying frequency, bandwidth, duration and rate (Kroodsma and Byers 1991; Slabbekoorn and den Boer-Visser 2006; Ríos-Chelén et al. 2013). Adaptive song alterations may occur via the preferential learning of certain song types or via the flexible use of certain song types and modification of song form with context (Tumer and Brainard 2007; Bermúdez-Cuamatzin et al. 2009; Ríos-Chelén et al. 2012; Grabarczyk, Vonhof and Gill 2019). Evidence shows that song-learning birds are better than non-learners at adjusting their vocalizations in response to environmental changes due to urbanization (Ríos-Chelén et al. 2012).

Urban environments can shape acoustic signals in variable ways. Multiple studies have reported that songs of urban birds have higher minimum frequencies when compared with non-urban bird populations (Gil and Brumm 2013). Because low-frequency songs propagate better than high-frequency songs in closed habitats (Boncoraglio and Saino 2007; but see Karin et al. 2018), we might predict that songs with dominant low frequencies would be successful in urban environments where infrastructure creates an abundance of clutter and reflective surfaces (Phillips et al. 2020). However, urban birds are competing with anthropogenic sounds such as cars that dominate the low-frequency soundscape; this provides a logical explanation as to why several species have been observed to raise their song minimum frequencies in urban areas, thereby avoiding being masked by anthropogenic noise (Derryberry et al. 2020). Still, scientists have predicted (and studies show) that birds modify several components of their songs differently in response to urban environments (Table 1). The varied and sometimes conflicting results reported in the literature (Table 1) suggest that changes to song elements may be specific to species, population or context (Hu and Cardoso 2010; Derryberry et al. 2020).

Changes to song form resulting from neutral cultural evolution over time and space are less predictable than changes resulting from habitat alteration but may be equally important in influencing signal evolution. Additionally, changes to song form via cultural drift coupled with ecological selection may affect different parts of a species' song in different ways. Often, introductory phrases in bird songs alert listeners to the species identity of the singer, while later phrases carry information about individual identity, population affiliation, quality or other aspects of individuality (Richards 1981; Cicero and Benowitz-Fredericks 2000; Nelson 2017). Song components important in species identification should remain stable through time, while song components that signal individual attributes have more flexibility to vary with local culture (Benedict and Bowie 2009). Thus, different song phrases may show distinct evolutionary patterns over space and time due to their different functions (Williams et al. 2013; Roach and Phillmore 2017; Lee, Podos and Sung 2019). Additionally, cultural drift could lead to geographic gradients in one or both song parts if they are not subject to strong selection imposed by urbanization (Benedict and Bowie 2009; Ramsay and Otter 2015).

The spotted towhee (*Pipilo maculatus*) is a widespread and abundant species of western North America. Spotted towhees are found commonly on the edges of cities and are facing population threats due to urban sprawl and human development (Bartos Smith and Greenlaw 2020). The songs of males are relatively simple and consist of a pure-toned pre-trill or whistle and a trill phrase (Fig. 1). Songs from individuals in some parts of the range also include an introductory phrase. Evidence of dialects in some subspecies, and the presence of local and regionally shared song elements, suggest that males learn songs as young individuals in their natal populations (Roberts 1969; Kroodsma 1971; Borror 1975). Previous studies of spotted towhee songs have demonstrated geographic and individual-level variation in the use of introductory phrases and other song features (Kroodsma 1971; Borror 1975), suggesting the potential for cultural evolution in song structure. Additionally, Richards (1981) found evidence that these songs are separated into alerting and messaging components, with the introductory phrase and pre-trill serving as an alert while the trill phrase encodes a message. The clear evidence of cultural transmission of flexible song elements suggests that this species has the potential to show marked song variability during urbanization events. Like many other species, spotted towhees are known to adjust song parameters and behavioral responses in noisy environments (Francis et al. 2012; Kleist et al. 2016). Although this existing research has examined noise associated with oil and gas

Table 1. Examples of some bird song responses to urbanization presented in the scientific literature. Studies included in this table represent song responses of several different species. This table is not exhaustive and does not include studies where no change was found in songs.

Song characteristic	Response	Example studies
Minimum frequency	Increase	Slabbekoorn and den Boer-Visser 2006; Wood and Yezerinac 2006; Bermúdez-Cuamatzin et al. 2009; Dowling, Luther and Marra 2012[a]; Redondo, Barrantes and Sandoval 2013; Job, Kohler and Gill 2016; Derryberry et al. 2020
Minimum frequency	Decrease	To, Dingle and Collins 2021
Maximum frequency	Increase	Dowling, Luther and Marra 2012; To, Dingle and Collins 2021
Maximum frequency	Decrease	Dowling, Luther and Marra 2012
Peak frequency	Decrease	Job, Kohler and Gill 2016; To, Dingle and Collins 2021
Duration	Increase	Nemeth and Brumm 2009; Verzijden et al. 2010; Francis et al. 2012; Moseley et al. 2019
Duration	Decrease	Slabbekoorn and den Boer-Visser 2006; Nemeth and Brumm 2009; Francis et al. 2012; Hill et al. 2018; Cyr et al. 2020
Syllable/trill rate	Increase	Redondo, Barrantes and Sandoval 2013; Job, Kohler and Gill 2016; Cyr et al. 2020
Bandwidth	Increase	Lohr, Wright and Dooling 2003; Wood and Yezerinac 2006; Dowling, Luther and Marra 2012; Gil and Brumm 2013; Redondo, Barrantes and Sandoval 2013
Bandwidth	Decrease	Dowling, Luther and Marra 2012; Job, Kohler and Gill 2016

[a]Dowling, Luther and Marra (2012) found evidence of differing responses based on species; thus, we have included this citation in several response categories.
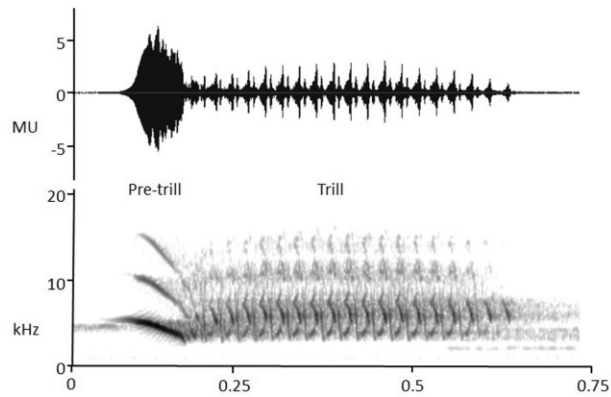
Figure 1. Waveform and spectrogram of a spotted towhee (*Pipilo maculatus*) song from California, illustrating the pre-trill, and trill phrases. MU on the *y*-axis of the audio waveform represents Raven Pro 1.4 amplitude units.

development rather than with urbanization, both sources of noise occupy low-frequency space, likely impacting this species in similar ways.

Research aims

To better understand how urban spaces alter bird songs, it is valuable to sample changes to multiple song components over time in developing urban areas. Further, it is important to implement methods that capture variation in song and habitat components while they are changing in urbanizing landscapes. With that goal in mind, we report here on changes in spotted towhee songs through space and time following a period of urban growth. Specifically, we analyzed song recordings of spotted towhees from two time-points separated by 45 years (1970, 2015) in the San Francisco Bay Area. We examined spotted towhee pre-trill and trill song phrases separately to assess whether different song elements are evolving on separate trajectories. In general, we hypothesized that as time passed and development increased, birds would adjust their songs to increase signal transmission in noisy urban environments by increasing their minimum frequency and consequently decreasing bandwidth, as has been found in many other species (Gil and Brumm 2013). We also predicted that duration and trill rate would increase over time and with increased development to increase the likelihood of the song messaging component being heard by the receiver. If songs are heavily shaped by habitat, we predicted that both the pre-trill and the trill components would be affected by development and time, resulting in similar directional shifts of song characteristics. Alternatively, if stabilizing selection is important for species recognition and is stronger than the impacts of habitat, then we predicted that the pre-trill (the 'alerting' component) would be more stable over time, while cultural evolution would allow for more adjustment of the trill ('messaging' component) phrase of the song (Richards 1981).

Methods

Study populations

Our dataset included songs of male spotted towhees from seven populations in northern California. Sampling sites covered a distance of nearly 162 km east-northeast from the San Francisco Bay Area to the Sierra Nevada foothills (Fig. 2). Levels of urbanization generally decrease from western to eastern populations along this transect.
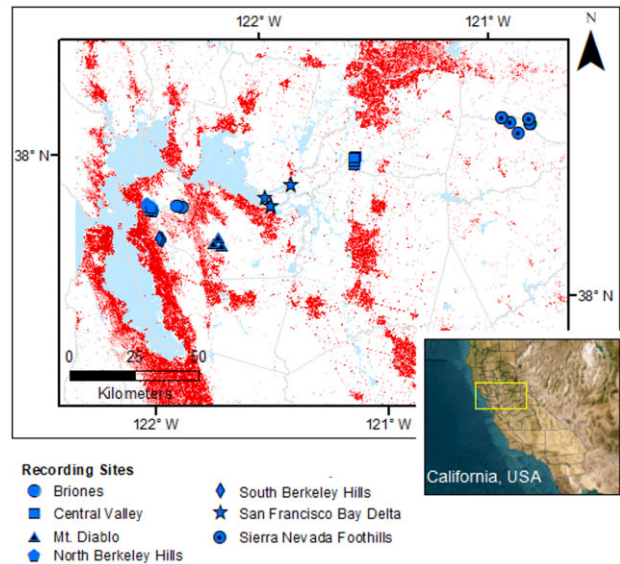
Figure 2. Recording locations for spotted towhees (*P.maculatus*) in northern California in 1970 and 2015 with county outlines in gray. Each site had several recording locations within the area. Sites are grouped by shape. Developed land is shown in red, water is shown in blue and all other land cover types are presented in white.

Song recordings

Songs were recorded in 1970 and again in 2015 along the same transect. The 1970 analog recordings were obtained by Susan Kishler and digitized to WAV files by the UC Berkeley Language Center using 128 kb/s precision at a 4800 Hz sampling rate, uncompressed, with a mono setting. Field and recording notes from the 1970 dataset were used to target locations for the 2015 recordings. Songs from 2015 were recorded by one of us (K.F.) using a Sennheiser shotgun microphone and a Marantz PMD 661 or PMD 670 digital recorder set at a 4400 Hz sampling rate, 24-bit and WAV file type. All recordings were taken during the breeding season (March–August). Because individuals were not banded in 2015, birds in adjacent territories were recorded only if both individuals could be heard singing simultaneously. Otherwise, the recordist moved at least 400 m along the transect before recording any other individuals to assure independent sampling. Individuals were recorded for as long as the bird continued singing or until 40 songs were reached. Whether individuals were banded in 1970 is unknown due to a lack of archived data to describe these details. Although recording equipment varied between the two time-points, comparisons should be valid because research has demonstrated that differences in historical recording approaches and devices do not significantly affect the integrity of spectral features of songs (Derryberry 2007; Luther and Derryberry 2012; Moseley et al. 2019). Songs from both time periods are archived in the Museum of Vertebrate Zoology at the University of California, Berkeley (see Supplementary Resource S1 for catalog information).

We inspected recordings acoustically and visually by spectrogram using Raven Pro 1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology 2012) and Audacity (https://audacityteam.org) version 2.3.3. High-quality songs were chosen for analysis. A song was considered high quality if it had the following features: the entire song was clearly visible on a spectrogram with no overlap from other sounds, the waveform clearly indicated the timing of song elements and the recordist

indicated that the vocalization came from the target individual. Three high-quality songs were selected and measured for 95% of individuals. When three high-quality songs were not available, we selected two songs for measurement. Recordings were clipped to include only the selected songs and normalized to −1 dB. Songs were high-pass filtered at 1200 Hz to reduce noise below the frequency of spotted towhee vocalizations and saved as WAV files using audacity. Our analysis included 435 songs from 148 individuals in the 1970 dataset and 364 songs from 123 individuals in the 2015 dataset, for a total of 799 songs analyzed (Table 2).

Song characteristics

Introductory phrases were uncommon in the dataset and therefore we focused our analyses on the pre-trill and trill phrases. We visualized spectrograms in Raven Pro 1.5 using Hanning-type spectrograms with a discrete Fourier transform of 512 samples, a frequency resolution of 86.1 Hz and a time resolution of 5.8 ms. From spectrograms, we measured the following song characteristics for each pre-trill and trill phrase: minimum frequency, maximum frequency, bandwidth, duration and trill rate. Because many of our recordings came from sites with background noise, we measured frequency features using the peak frequency contour (PFC) tool (Ríos-Chelén et al. 2017). This approach traces the contour of the song by identifying the frequency of peak amplitude at each spectrogram time-step. Therefore, our reported minimum and maximum frequency measures are the PFC minimum and PFC maximum, which should be consistent across background noise conditions. Values for PFC bandwidth were obtained by subtracting the PFC min from the PFC max. To measure phrase durations, we marked start and end times for the pre-trill and trill phrase using visual inspection of waveforms. We used the 90% duration measurement to measure duration for each portion of songs; 90% duration uses the power of the song to determine the length of 90% of the selected song area with the most power. This measure reduces the possibility of human error that come from manually drawing boxes around the length of songs. To calculate trill rate, we divided the total number of trill elements by the 90% trill duration. Trill elements were counted twice for each trill by close

Table 2. Sampling locations and sample sizes of spotted towhee (*Pipilo maculatus*) males and songs included in this study. Sites are listed from west (more urban) to east (less urban).

Site	Year	N males	Mean # songs/male	Total songs
North Berkeley Hills	1970	53	2.92	155
	2015	18	3	54
South Berkeley Hills	1970	10	2.9	29
	2015	9	3	27
Briones	1970	7	2.86	20
	2015	7	2.86	20
Mount Diablo	1970	5	2.8	14
	2015	22	2.9	64
San Francisco Bay Delta	1970	34	2.94	100
	2015	23	2.96	68
Central Valley	1970	15	3	45
	2015	23	3	69
Sierra Nevada Foothills	1970	24	2.96	71
	2015	21	3	63
Total		270	2.94	799

examination of waveforms. All song measurements were taken by the same observer (K.S.). Because frequency is non-linear in avian perception of sound, we log-transformed PFC minimum and maximum measurements prior to analysis (Cardoso 2013).

Landscape analysis

Recording locations from 1970 were determined based on verbal notations in recordings along with accompanying recording notes. Recordings taken in 2015 were collected as close as possible to sites that were described in the 1970 dataset and were georeferenced at the time of recording using a Garmin Global Positioning System unit.

We used the U.S.G.S. Modeled Historical Land Use and Land Cover 1970 layer to analyze land cover in 1970 (Sohl et al. 2016). Layers from the Multi-Resolution Land Consortium's National Land Cover Data 2016 were used for 2015 recording locations (Yang et al. 2018). Spotted towhees typically establish territories 1-ha in size (Mans 1961; Kingery 1962); thus, we created 1-ha buffer polygons, around recording locations for each of the 271 birds sampled using the Graphic Buffer tool with a square cap type and a miter joint type in ArcMap version 10.6.1. To extract land cover data within these polygons, we converted the hectare buffers to raster layers using the Feature to Raster tool. Land cover characteristics were then extracted from these layers with the Combine Spatial Analyst tool. For each hectare, the percentage of developed land was calculated by taking the number of cells within the hectare with a 'developed' score and dividing it by the total number of cells. The two land cover data layers differ in how they score developed land. The 2016 layer has four categories of development that differ by intensity of infrastructure, impervious surface and human population. The 1970 data have only one category that encompasses all developed land. Therefore, we chose to treat all development as a single category to avoid differences in scoring of degrees between layers. All analyses were done in the NAD 1983 State Plane, California III projected coordinate system. Data were compared visually against available Landsat imagery to check for errors that may have occurred in land characterizations.

Statistical analysis

We compared mean levels of development at sites in 1970 versus 2015 using a Wilcoxon signed rank test. Assumptions of normality and homoscedasticity in the response variables were verified by inspecting histograms. Pre-trill duration and trill rate were not normally distributed and were corrected using a log transformation prior to analysis. To test for changes in pre-trill and trill form over time and space, we constructed nine separate linear mixed models (LMMs) on nine separate song traits: four models to test for time and landscape effects on pre-trill characteristics and five models to test for effects on trill characteristics. We used the following measured characteristics as response variables for the pre-trill: Log PFC min, Log PFC max, PFC bandwidth and 90% duration. We constructed a similar set of LMMs for the trill phrase, but also included one for the response variable trill rate. In all models, we included year (1970, 2015) and percent developed land as factors with fixed effects. To test for the crossed effect of year and developed land, we included an interaction between those two variables. To assess multicollinearity, we reviewed VIF scores of each model and found little collinearity among our predictor variables; therefore, all were included in the models (all scores <1.4). Furthermore, we nested 'Individual' within 'Site' and included this as a factor with

random effects to account for repeated measures of individuals and variable singing behavior by individual birds.

We built nine models in total and used a Holm–Bonferroni correction for multiple comparisons for the pre-trill models and for the trill models (Holm 1979). Following each model, we plotted residuals with explanatory variables to assess the fit of the model and plotted the residuals against the fitted values to determine if there was non-constant error variance. Statistical analyses were done in JMP® (SAS Institute Inc. 2019).

Results

Landscape analysis

Developed land at our sampling sites increased from a mean of 2% to 21% between 1970 and 2015, with all sites experiencing significant increases ($P < 0.001$, $\alpha = 0.05$, Table 3). Percent development in the hectares surrounding individual recording locations ranged from 0% to 100% in 1970 and 0% to 70% in 2015. In both years, the South Berkeley Hills site had recording locations with the highest levels of development and the Briones site had recording locations with the least developed land. While Briones had the largest change in developed land from 1970 to 2015 (50% increase in developed land), it nonetheless had the least urban development in both time periods.

Song characteristics

Pre-trills: LMMs indicated that the level of urban development within a single year did not predict any pre-trill song characteristics tested. Time had mixed effects on pre-trill form. While duration, PFC minimum frequency and PFC maximum frequency were not significantly affected by year, our models suggested that year did predict pre-trill bandwidth. Songs in 1970 had significantly wider pre-trill bandwidths than 2015 songs ($F = 8.267$, $P = 0.0035$, $\alpha = 0.0041$; for full model results, see Table 4 and Fig. 3).

Trills: As with pre-trills, percent developed land alone did not predict within-year variation in any of the trill characteristics measured. Year did predict duration such that trills from 1970 were significantly shorter than trills from 2015 ($F = 12.035$, $P = 0.0006$, $\alpha = 0.0041$), but year did not have an effect on PFC minimum frequency, PFC maximum frequency, bandwidth or trill rate. The strongest effects detected by our models were interactions between year and land development on trill characteristics. In all cases, the interaction effect of developed land and year revealed opposite directional relationships (Figs 3 and 4). In 1970, the percent of developed land was associated with an increase in trill PFC maximum frequency and PFC bandwidth. Conversely, these characteristics decreased with development in 2015 songs. The interaction effect of year and

development was found to be significant in the PFC maximum model (PFC max frequency: $F = 14.1$, $P < 0.0002$, $\alpha = 0.0038$; for full model results, see Table 4).

Discussion

We analyzed songs of spotted towhees across a transect with varying levels of urban development to assess differences in song characteristics between two time-points separated by 45 years (1970 and 2015). Although the percentage of developed land increased significantly across all sites, this variable alone did not have consistent and significant effects on song characteristics as predicted. When combined with time, a significant crossed effect revealed that song features covaried differently with development in 1970 and 2015. Additionally, we found that trills showed more differences with urban development and time than did pre-trills.

Songs from 1970 and 2015 responded differently to development

Following current literature, we predicted that both the passage of time together with an increase in the level of urban development at each time period would lead to increases in spotted towhee song frequencies, duration and trill rate (Slabbekoorn and den Boer-Visser 2006; Francis et al. 2012; Derryberry et al. 2016). Interestingly, we did not find a clear consistent directional change to song form that matched these predictions. Minimum frequency, which appears to be the song feature most affected by noise in previous research (Slabbekoorn 2013; Sol, Lapiedra and González-Lagos 2013; Reichard et al. 2020), showed no consistent variation with either the level of urban development or time (Figs 3 and 4). As predicted, birds recorded in 2015 in more urban areas tended to sing trills with narrower bandwidths. However, these narrow bandwidths in urban areas were not a result of higher minimum frequencies but rather lower maximum frequencies.

One of our strongest and most surprising findings is that birds from 1970 and 2015 had songs features that covaried inversely with urbanization. Specifically, trill maximum frequency was significantly predicted by the crossed effect of year and developed land. Trill bandwidth also closely followed this trend, although the result only approached statistical significance ($F = 8.016$, $P < 0.0046$, $\alpha = 0.0045$; Table 4). These results reveal opposite directional effects of developed land on maximum frequency and bandwidth within each year. In contrast to 1970, spotted towhees recorded in 2015 had reduced pre-trill bandwidths and trill bandwidths narrowed as development increased. This strategy could potentially allow individuals to cut through urban noise better or reduce attenuation in

Table 3. Measured mean (SE) percent developed land for all spotted towhee (*P.maculatus*) recording sites at two sampled time points.

Site	Mean % developed land (SE)		Z-statistic	P-value
	1970	2015		
North Berkeley Hills	11 (0.02)	11 (0.02)	5.86	<0.0001
South Berkeley Hills	11 (0.05)	38 (0.02)	5.35	<0.0001
Briones	0 (0)	10 (0.002)	5.88	<0.0001
Mt. Diablo	0 (0)	32 (0.008)	−6.10	<0.0001
San Francisco Bay Delta	0 (0)	34 (0.02)	12.47	<0.0001
Central Valley	0 (0)	12 (0.01)	−6.69	<0.0001
Sierra Nevada Foothills	0 (0)	25 (0.02)	8.95	<0.0001

Table 4. LMM results for song characteristics. Significant effects following Holm–Bonferroni corrections are in bold.

|		Estimate (SE)	F ratio	P-value	Lower 95% CI	Upper 95% CI
Pre-trill measurements						
Log PFC Min	Intercept	8.07 (0.02)	343.16	<0.0001	8.02	8.12
	Year (1970)	−0.03 (0.02)	−1.68	0.093	−0.07	0.01
	% Developed area	0.03 (0.09)	0.32	0.750	−0.15	0.21
	Year × developed area	0.23 (0.09)	2.65	0.0162	0.05	0.41
Log PFC Max	Intercept	8.41 (0.01)	558.20	<0.0001	8.38	8.44
	Year (1970)	0.01 (0.01)	1.23	0.220	−0.01	0.04
	% Developed area	0.05 (0.058)	0.78	0.434	−0.07	0.16
	Year × developed area	0.14 (0.058)	2.38	0.018	0.02	0.25
PCF bandwidth	Intercept	1247.78 (65.8)		<0.0001	1118.2	1377.3
	Year (1970)	**145.71 (50.7)**	**8.267**	**0.0035**	**45.93**	**245.49**
	% Developed area	136.80 (252.8)	0.293	0.5888	−360.88	634.49
	Year × developed area	−18.91 (252.8)	0.006	0.9404	−516.59	478.77
Log 90% duration	Intercept	−3.30 (0.05)		<0.0001	−3.39	−3.20
	Year (1970)	0.00 (0.04)	0.01	0.9193	−0.07	0.07
	% Developed area	0.22 (0.19)	1.40	0.2382	−0.15	0.59
	Year × developed area	0.04 (0.19)	0.04	0.8521	−0.33	0.40
Trill measurements						
Log PFC Min	Intercept	5.50 (1.3)	4.85	<0.0001	3.27	7.73
	Year (1970)	0.00 (<0.001)	2.32	0.021	<0.01	<−0.01
	% Developed area	0.05 (0.06)	0.76	0.447	−0.08	0.17
	Year × developed area	−0.00 (0.002)	−0.89	0.374	<−0.01	<−0.01
Log PFC Max	Intercept	9.34 (0.74)	12.7	<0.0001	7.90	10.79
	Year (1970)	−0.00 (<0.001)	−0.75	0.453	<−0.01	<−0.01
	% Developed area	−0.07 (0.04)	−1.60	0.111	−0.15	0.02
	Year × developed area	**−0.01 (<0.001)**	**−3.71**	**<0.0002**	**−0.01**	**<−0.01**
PCF bandwidth	Intercept	3124.65 (76.0)		<0.0001	2974.9	3274.4
	Year (1970)	117.95 (58.6)	4.05	0.0453	2.48	233.42
	% Developed area	−650.53 (293.2)	4.92	0.0273	−1227.7	−73.33
	Year × developed area	830.02 (293.2)	8.02	<0.0046	252.81	1407.22
Log Trill rate	Intercept	3.67 (0.05)		<0.0001	3.57	3.76
	Year (1970)	0.04 (0.04)	1.31	0.2539	−0.03	0.11
	% Developed area	0.01 (0.18)	0.00	0.9551	−0.34	0.37
	Year × developed area	−0.40 (0.18)	5.039	0.0256	−0.76	−0.05
90% duration	Intercept	0.58 (0.009)		<0.0001	0.57	0.60
	Year (1970)	**−0.03 (0.007)**	**12.04**	**0.0006**	**−0.04**	**−0.01**
	% Developed area	−0.04 (0.04)	1.26	0.2634	−0.11	0.03
	Year × developed area	0.05 (0.04)	1.53	0.2116	−0.03	0.12

closed urban habitats if it allows for birds to concentrate more energy in particular frequencies, thereby allocating acoustic energy to increase amplitude (Phillips et al. 2020). Urban land cover increases have been shown to drive down maximum song frequencies in some other species (Dowling, Luther and Marra 2012), but it is still unclear if this pattern has adaptive significance.

The documented patterns of song variation in our study populations of spotted towhees suggest a good deal of flexibility in song form in this species. Previous studies have suggested that random variation and cultural drift might cause change over time and across sampled geographic gradients. Spotted towhee songs are relatively high-pitched compared with other urban species (Dowling, Luther and Marra 2012; Francis et al. 2012). Typically, most anthropogenic noise is found at or below 3000 Hz (Wood and Yezerinac 2006; Goodwin and Shriver 2011), although it can go to higher frequencies. Spotted towhee songs are known to be just slightly above 3000 Hz (Figs 3 and 4), suggesting that their whole songs may not be competing with anthropenic noise. In a prior study, Richards (1981) showed that higher frequency spotted towhee trills did not improve response latencies in conspecifics, suggesting that high frequencies are not necessary for recognition. Kleist et al. (2016) showed

that noise negatively impacts response latencies to song in this species, potentially placing a strong selective pressure on the ability to be heard, but they did not indicate which song elements were heard best. Together, these results indicate that spotted towhee song transmission is important but that the loss of high frequencies in their songs might have little effect on signal efficacy. Thus, there could be room for variation in this trait over space and time that is not strongly driven by adaptation to urban environments. This contrasts with the trend seen in species with lower-pitched songs, which show stronger masking effects in urban spaces (Lohr, Wright and Dooling 2003; Dowling, Luther and Marra 2012; Phillips et al. 2020).

## Song components responded differently to development and time

We hypothesized that the variability associated with cultural evolution of song would lead to more changes in song elements associated with individuality (trill phrase) than species-identity signaling (pre-trill). In support of this, we observed more change in trill phrases than in pre-trills over space and time. Our
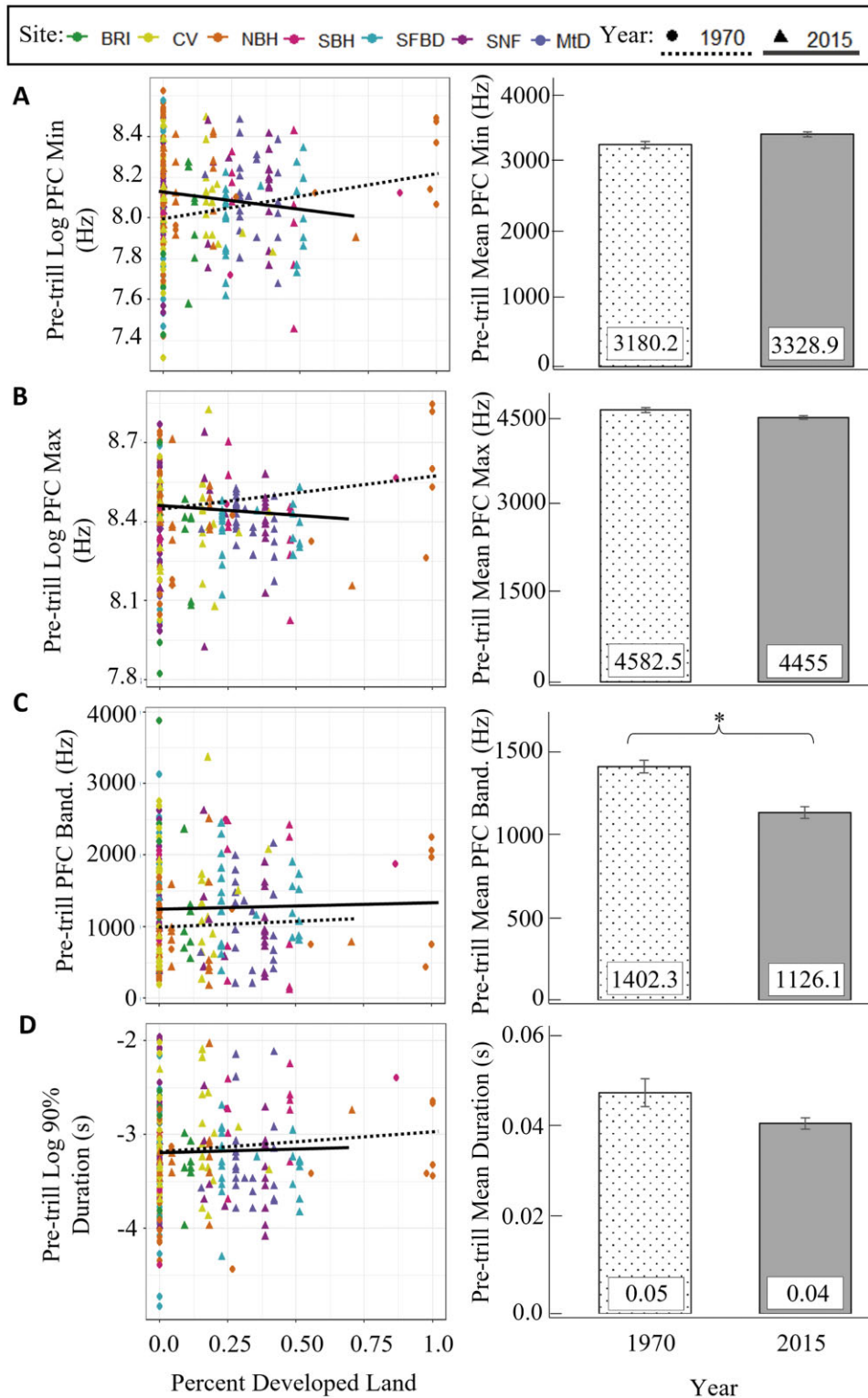
Figure 3. Linear relationships of percent developed land and song pre-trill characteristics for songs recorded in 1970 (circles) and 2015 (triangles) are found on the left. Recording location is indicated by color. Means and standard error for each song characteristic measurement by year are shown on the right in pre-transformed units for better interpretation of measurements. Significant predictors following the Holm–Bonferroni correction within each LMM are designated using an * at their respective alpha levels. Significant crossed effects of developed land and year are shown on the left, significant effects of year are shown on the right.

models revealed minor variation in pre-trills, with the only significant effect being that 1970 songs had wider pre-trill bandwidths than 2015 songs. Trills, in contrast, showed significant

variation in the interactions between years' maximum frequency, and duration, suggesting that evolution may be acting differently on song parts with different functions.
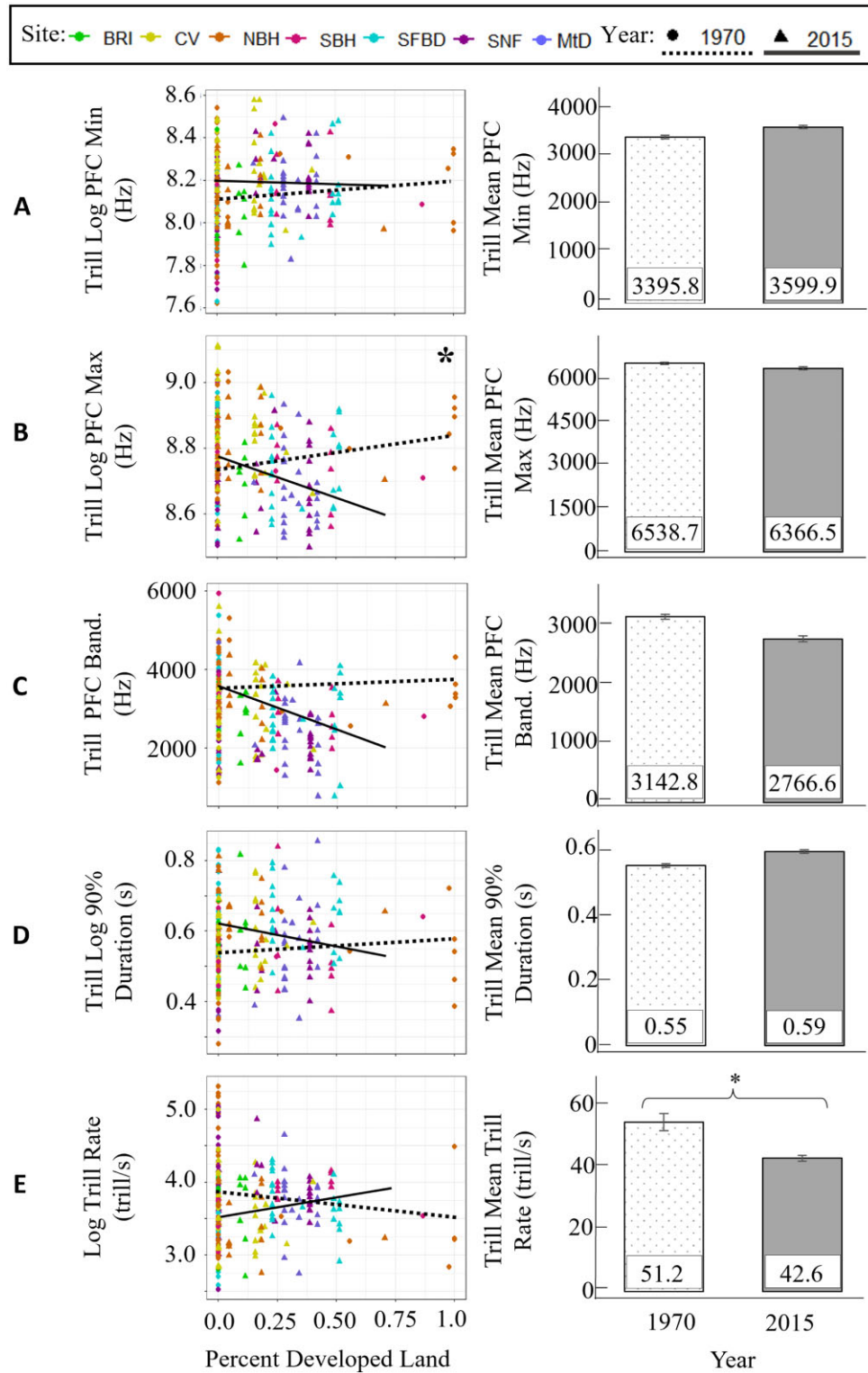
Figure 4. Linear relationships of percent developed land and song trill characteristics for individual songs recorded in 1970 (circles) and 2015 (triangles). Recording location is indicated by color. Means and standard error for each song characteristic measurement by year are shown on the right in pre-transformed units for better interpretation of measurements. Significant predictors following the Holm–Bonferroni correction within each LMM are designated using an * at their respective alpha levels. Significant crossed effects of developed land and year are shown on the left, significant effects of year are shown on the right.

Theory suggests that the 'message' component of a song can vary over space due to local social or habitat conditions, while the 'alert' portion of the signal should be under stabilizing selection for signaling species or population identity (Richards 1981; Nelson, Hallberg and Soha 2004; Williams et al. 2013; Williams 2021). Here, the trill is the 'messaging' component. When changes in trill phrase structure show plastic responses to current conditions (Halfwerk and Slabbekoorn 2009; Gross,

Pasinelli and Kunc 2010; Bermúdez-Cuamatzin et al. 2011), these changes will likely be passed on to young learners at different rates. If changes to song structures are necessary to transmit through an environment, those that break free from being masked by anthropogenic noise will likely reach a receiver more often. Thus, these altered songs are expected to be copied more frequently by first-year individuals (Luther and Baptista 2010; Slabbekoorn 2013), although recent work suggests that young individuals may learn songs equally during quiet times of the day (Liu, Zollinger and Brumm 2021).

The changes that we observed in spotted towhee songs over space and time did not conform to widespread predictions of urban ecology, suggesting that spatio-temporal changes may be a response to local social pressures and not driven by habitat (Williams 2021). In this study, we observed a switch from one trill structure to another between 1970 and 2015. Within the 45 years between our two time periods, it is likely that several other strategies for singing in urban areas were present and ultimately the strategy seen in 2015 was learned more often (Gomes, Francis and Barber 2021). Because we did not see a direct significant effect of land development alone on any of the song characteristics measured here, the observed patterns could be due to cultural drift within populations along the geographic range sampled. Unfortunately, we did not have enough song samples from each population within each year to adequately test this theory. The fact that trills changed more than pre-trills in multiple features lends support to the idea that drift, rather than specific impacts of urbanization, is creating the observed patterns. Future longitudinal studies like ours, with more frequent sampling, would help to evaluate the power that plastic behaviors and their interactions with the environment may have on cultural selection or drift within urban populations.

Conclusions

Spotted towhees are one of the many bird species that have been observed with altered songs in noisier areas associated with anthropogenic changes (Francis et al. 2012). Existing evidence of local dialects and song variation in spotted towhees suggests that both long-term cultural evolution and behavioral change act on the evolution of song structure in this species (Kroodsma 1971; Borror 1975; Francis et al. 2012). While our data do not allow us to tease apart the separate effects of selective song learning versus plastic vocal adjustment, it is likely that both contributed to the patterns we documented relating urbanization to song features at two time-points. Our results confirm that signals show variable patterns relative to human land cover change, and highlight open questions about how new song forms may be generated and perpetuated in a population. Changes in song can alter sexual selection and species recognition, ultimately influencing species' evolutionary trajectories (Slabbekoorn and Smith 2002). Human development can generate pressures that guide song evolution, but spotted towhees do not seem to show expected song adjustments consistently in areas of urban development. At some point between 1970 and 2015, spotted towhees in urban areas shifted from one song form (with increased maximum frequency) to another (with decreased maximum frequency). This change could reflect adaptations to improve signal transmission in new signaling environments, but it also could reflect cultural drift or social selection. Either way, it highlights the remarkable flexibility of bird song over time, the relatively rapid shifts in trait values across nearby populations and the unpredictability of urban habitat changes on bird song. Open questions remain about how often song traits might reverse their directional trajectories through evolutionary time in species that experience changing habitats.

Declarations

Ethical approval

Author contributions

Karina A. Sanchez (Conceptualization equal], Data curation [equal], Formal analysis [lead], Investigation [lead], Methodology [lead], Project administration [lead], Visualization [lead], Writing—original draft [lead]), Kristina Fialko (Conceptualization [equal], Data curation [equal], Investigation [equal], Methodology [supporting], Writing—review and editing [supporting]), Lauryn Benedict (Conceptualization [equal], Data curation [equal], Methodology [equal], Project administration [equal], Resources [supporting], Supervision [equal], Writing—review and editing [equal]), Carla Cicero (Conceptualization [equal], Data curation [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Supervision [equal], Writing—review and editing [lead]).

Supplementary data

Supplementary data are available at *JUECOL* online.

Conflict of interest statement. None declared.

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Data availability

Audio files used for this analysis are archived at the Museum of Vertebrate Zoology and accessible through the museum's on-line database Arctos (https://arctos.database.museum). A list of the cataloged tracks can be found in Supplementary Resource S1. Measurements of song characteristics are included in Supplementary Resource S2.

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