

# Stability and change through time in the dialects of a Neotropical songbird, the Rufous-collared Sparrow

Natalia C. García<sup>A,C</sup>, Ramiro S. Arrieta<sup>A</sup>, Cecilia Kopuchian<sup>A,B</sup> and Pablo L. Tubaro<sup>A</sup>

<sup>A</sup>División Ornitología, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Avenida Ángel Gallardo 470, C1405DJR, Buenos Aires, Argentina.

<sup>B</sup>Centro de Ecología Aplicada del Litoral (CECOAL) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ruta 5, km 2.5, 3400, Corrientes, Argentina.

<sup>C</sup>Corresponding author. Email: [ngarcia@macn.gov.ar](mailto:ngarcia@macn.gov.ar)

**Abstract.** Behavioural characters that are learned can show temporal variation resulting from changes introduced in new generations during the learning process. The song of the Rufous-collared Sparrow (*Zonotrichia capensis*) shows significant geographical variation associated with habitat, delimiting song dialects. To study the temporal stability or change in dialects of Rufous-collared Sparrows at a microgeographical scale, we compared recordings made at Reserva El Destino, in north-eastern coastal Buenos Aires Province, Argentina, during 1987 and 2000 with recordings made during 2010 and 2011. We found that the geographical boundaries of the dialects remained stable over the period of 24 years. As we did not detect gross changes in the habitat of the study area over that period, we conclude that habitat is an important factor in maintaining geographical boundaries of dialects. Within the dialects, variation in some variables detected between 1987 and 2000 persisted to 2010–11 whereas other variables showed reversal over that period. These findings are consistent with the hypothesis that some changes in song were the result of neutral variation, whereby changes resulting from random factors, such as copying error, could have been maintained or introduced across generations. Our results show that comparing more than two points in time is fundamental to a better understanding of temporal variation of song and its possible causes.

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## Introduction

Behavioural traits that are learned may change over time, similar to evolution in traits with a genetic basis. Variation in learned behaviours can arise within a generation through copying errors or innovation (analogous to mutations in genotype). Such variation can become fixed in a population if it is advantageous for the individual in some way (e.g. characteristics of avian songs may have increased transmissibility in particular habitats or be preferred by females) or the variation can be eliminated if it is deleterious. Variation in a trait that does not affect the fitness of an individual can also become fixed in a population (Lynch 1996). Vocal dialects – geographical variation in learned acoustic signals (see Mundinger 1983; Lynch *et al.* 1989) – have been described for a number of species of bird and these species are interesting models to investigate the processes responsible for stability or change in dialects. As well as variation between species, vocalisations within avian species can vary in space, between subspecies and between populations (reviewed in Podos and Warren 2007). However, there are fewer studies of temporal variation in vocalisations, because it requires long-term comparisons in a particular location (Catchpole and Slater 2008). To date, most studies of vocalisations over time have compared only two points in time. Although useful, such comparisons cannot determine if,

for example, change in a song variable has been directional or if it is randomly patterned (with increases and decreases between the time points).

Song is subject to several sources of selection, including ecological, social and perceptual conditions (Catchpole and Slater 2008). Changes in dialects across generations that are the consequence of changes to habitat over time can be expected when vocal dialects are strongly associated with habitat structure. This is considered the case in the Rufous-collared Sparrow (*Zonotrichia capensis*). The song of this species has two parts: an introductory theme and a final trill (Nottebohm 1969). The duration of the interval between consecutive notes of the trill – the trill interval – shows significant geographical variation and determines song dialects in this species (Nottebohm 1969, 1975; Handford and Loughheed 1991). There is a strong relationship between the trill dialect and vegetation type: trill intervals are often significantly longer in closed (forested) habitats than in open habitats (Nottebohm 1969, 1975; Handford 1981, 1988; but see Lijtmaer and Tubaro 2007 for an example where the opposite is true). Longer trill intervals may be better for long-range communication in forests, where the main source of signal degradation is reverberation from trunks and branches (Wiley and Richards 1982). Sound reverberation can fill short inter-note

spaces with echoes and complicate signal recognition by the listener (Wiley and Richards 1978, 1982; Richards and Wiley 1980). The dialects in this species are parsimoniously explained by different efficacies of transmission, and juvenile males are more likely to learn songs that have been less degraded by the environment (Hansen 1979; Lynch 1996).

In the Aconquija Mountains of north-western Argentina, Handford (1988) found that the geographical distribution of dialects had been stable for ~20 years and inferred much longer stability (~200 years) based on the fit of dialect distribution with the original distribution of habitat in the region compared with the present distribution of habitat (Handford 1988; Handford and Loughheed 1991). Lijtmaer and Tubaro (2007) studied dialects of Rufous-collared Sparrows in espinal woodland and steppe surrounding Guatraché Lake, in La Pampa Province, Argentina, and in an area of woodland that had been converted to grassland during the last century. The trill structure of the dialect in the modified area was intermediate between that of the dialects in the unmodified habitats. Lijtmaer and Tubaro (2007) suggested that song structure had changed after modification of habitat but could not confirm this because there were no recordings preceding the modification of habitat.

Kopuchian *et al.* (2004) compared recordings of Rufous-collared Sparrow songs from north-eastern coastal Buenos Aires Province, Argentina, made in 2000, with a set of recordings made 13 years earlier in 1987 (Tubaro *et al.* 1993). In the earlier study, there was a strong relationship between trill features of songs and vegetation type along a transect extending 7 km inland from the bank of the Río de la Plata (Tubaro *et al.* 1993). The Rufous-collared Sparrow songs from the open habitats in the transect have significantly shorter trill intervals than those from the closed habitats, defining two dialects. Kopuchian *et al.* (2004) analysed two aspects: the geographical stability of dialects (change in the geographical boundaries of dialects) and the stability of acoustic properties within each dialect. They found that the geographical boundaries of the dialects remained stable, a result congruent with the lack of gross changes in the habitat characteristics they examined. However, they also found changes in acoustical properties within each dialect. They suggested three main explanations for these results: that there had been subtle changes in habitat that they did not detect and that could have acted as a selection pressure on song evolution; that temporal variation in song structure was a response to past changes in the environment and so not detectable in their study; or that the changes were the result of neutral change as a result of copying errors over time.

We returned to the same transect in north-eastern coastal Buenos Aires Province in 2010 and 2011 and recorded songs of Rufous-collared Sparrows. We increased the overall span of analysis of change in dialects and also added a third time point to the comparison. This allows significantly better understanding of changes in song across generations, and its causes. The addition of a third point in time can allow determination of changes that were previously detected and whether any have reverted, which might point to random change.

The objectives of our study were to: (1) assess the association between dialects and habitat structure that had been described previously (Tubaro *et al.* 1993; Kopuchian *et al.* 2004); and (2) to determine if the differences in song characters detected by Kopuchian *et al.* (2004) had increased, remained stable or

reverted. The first two options (increase or stable) would be possible under any of two scenarios: undetected changes in the environment (actual or past) acting as pressures to change song, or neutral change. However, if reversions in characters were detected, we could reject the hypothesis of variation in song being adaptive and only the null hypothesis of neutral change would stand. We also tested if trill interval correlated with trill bandwidth (the difference between the maximum and minimum frequencies) as found previously for other populations of this species (Handford and Loughheed 1991). Changes in one aspect of song can result as a by-product of variation in other song-traits, owing to morphological or physiological constraints that may link them.

## Methods

### Study area

All the recordings analysed were made at Reserva El Destino, Partido de Magdalena, in north-eastern coastal Buenos Aires Province, Argentina (35°05'S, 57°31'W). The study area comprised a transect 1 km wide and extending 7 km inland from the banks of the Río de la Plata. The study site is a private reserve in which the natural habitats are reasonably well preserved.

Within the study area, three main types of habitat were distinguished: marshland (from the river banks to ~0.4 km inland), talar woodland (0.4–2.5 km inland from the river) and steppe grassland (2.5–7 km inland). The marsh is predominantly seasonally flooded lowland grassland, with areas of higher ground, abutting the Río de la Plata and dominated by *Scirpus*, *Salicornia* and *Paspalum*. The talar woodland is a mix of xeromorphic woodland and strips of grassland that run parallel to the Río de la Plata; the grassland strips are broader and more closely spaced near the marshland and farther apart inland, forming an ecotone with the inland steppe grassland. The talar woodland is dominated by tree species including *Celtis tala* (Ulmaceae) (spiny hackberry), *Scutia buxifolia* (Rhamnaceae), *Jodina rhombifolia* (Santalaceae) and *Acacia caven* (Fabaceae) (Roman-cassie), and has a dense understorey of shrubs and herbs. The steppe grassland is herbaceous open grassland dominated by species of *Stipa*, and with some shrubs but essentially no trees (with the exception of some planted rows of *Eucalyptus* spp. and a few isolated *C. tala*). For more detailed descriptions of these communities, see Parodi (1940) and Cabrera (1949, 1976).

### Song samples

We recorded songs of 184 Rufous-collared Sparrows over two breeding seasons: 27 in October 2010, 13 in October 2011, and 144 in November 2011, during 2–7 days each month. Because individuals were not banded, we attempted to ensure independence of data by not recording individuals within 50 m of a bird we had recorded, with the exception of neighbours singing simultaneously (Nottebohm 1969). Since Rufous-collared Sparrows generally sing only inside their territories, it is easy to sample the songs of an area without accidentally recording an individual more than once (Lijtmaer and Tubaro 2007). Given the small fraction of birds that were recorded in 2010, and considering that annual disappearance rate for adults ranges from 30 to 77.6% (Miller and Miller 1968; Handford 1980), it is also unlikely that we recorded the same individual in 2010 and 2011. Further, although singing Rufous-collared Sparrows are almost always

males (King 1972), because the sexes are monomorphic, it is possible that a small number of recordings are of females. Recordings were made with a Tascam HD P2 digital recorder (Teac America Inc., Montebello, CA) and a Sennheiser K6ME67 directional microphone (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany). A bird was recorded until it stopped singing or until a maximum of 10 complete songs was recorded, resulting in ~5–10 songs per individual. Because few birds were recorded in marshland, we pooled marshland and steppe grassland recordings into a single category, open habitat, and categorised samples from the talar woodland as closed habitat.

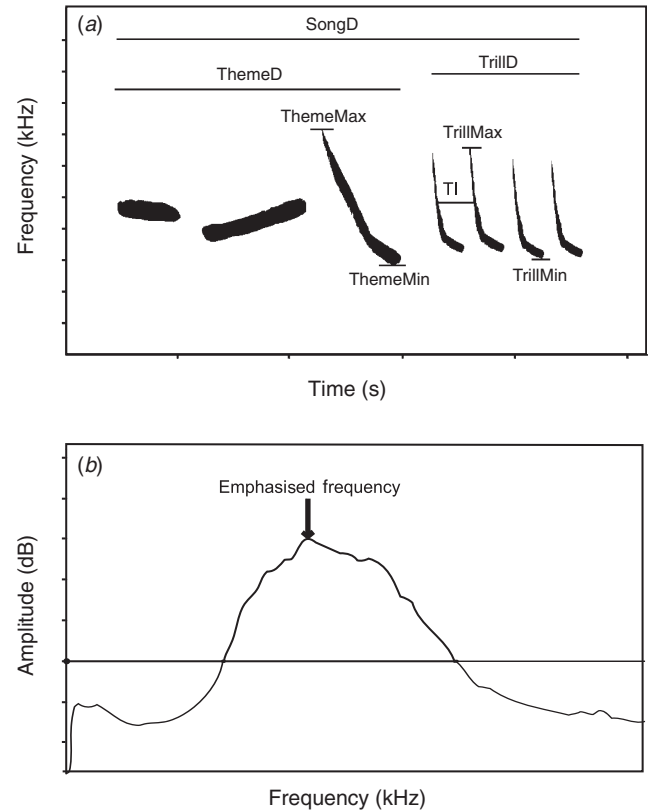
Individuals of this species sing only one song-type and show low variation between songs (Nottebohm 1969, 1975; Handford and Nottebohm 1976). Therefore, in most cases, we included only one song per individual in the analyses. The song was selected based mainly on sound quality. However, for individuals that sang two songs (<5%), we included one song of each type in our analyses. (Excluding these individuals from the analyses did not change the results.)

#### Analysis of songs

For each recording, a spectrogram was generated and the selected song was analysed using the software Raven, version 1.4 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY; see <http://www.birds.cornell.edu/raven>, accessed 9 October 2013). For spectrogram analysis, we used an FFT of 256 samples, the Hamming window function, a window size of 256 samples and ~75% brightness and contrast. The following time-related variables were measured for each song (Fig. 1a): duration of the song (SongD, in ms); duration of the theme (ThemeD, in ms); duration of the trill (TrillD, in ms); number of trill notes (NNotes); and duration of trill interval (TI, in ms, calculated as  $\text{TrillD} \div (\text{NNotes} - 1)$ ). TrillD was measured from the middle of the first note of the trill to the middle of the last note. We defined a note as a continuous trace on the temporal axis of a spectrogram. We also measured the following frequency-related variables: maximum and minimum frequencies of the whole song (SongMax and SongMin, in kHz), of the theme (ThemeMax, ThemeMin) and of the trill (TrillMax, TrillMin); and the frequency bandwidth for the trill (TrillBand, calculated as  $\text{TrillMax} - \text{TrillMin}$ ). Lastly, the emphasised frequency (i.e., the frequency with the highest amplitude) was measured on the power spectrum of the song (SongEmp), theme (ThemeEmp) and trill (TrillEmp) (see Fig. 1b).

#### Statistical analysis

We compared song parameters between dialects of each type of habitat (open and closed) for 2010–11 recordings using nonparametric Mann–Whitney *U* tests, because several variables did not meet assumptions of homoscedasticity. We compared our dataset to recordings made in 1987 and 2000, and analysed by Kopuchian *et al.* (2004). These previous recordings were performed using magnetic recording tape. We obtained the raw data from the analysis performed by Kopuchian *et al.* (2004), who measured the same variables as we did, and used it to perform a Kruskal–Wallis test to look for general differences between years in each type of habitat. For those variables that showed significant variation, we used Mann–Whitney *U* tests to examine differences



**Fig. 1.** (a) Spectrogram and (b) power spectrum of a typical Rufous-collared Sparrow song showing the variables measured. SongD, duration of song (ms); ThemeD, duration of theme (ms); TrillD, duration of trill (ms); TI, duration of trill interval (ms); ThemeMax and ThemeMin, maximum and minimum frequency of theme (kHz); TrillMax and TrillMin, maximum and minimum frequency of trill (kHz).

between our recordings and those from 1987 and 2000 (within a given habitat). Because we made multiple comparisons between groups using several variables, we used a Bonferroni–Holm procedure (Holm 1979) to correct the  $\alpha$  and keep the global type I error <5%. To test the strength of a relation between the variables TI and TrillBand we calculated the Pearson correlation coefficient (*r*) between these variables.

All statistical analyses were performed using the software Statistica, version 8.0 (StatSoft Inc., Tulsa, OK) and all tests were two-tailed.

## Results

Tables 1 and 2 provide descriptive statistics for the song variables that were measured in the three studies (1987, 2000 and 2010–11), and the results of the Kruskal–Wallis tests for differences between years, for each of the habitat categories: closed habitat (Table 1) and open habitat (Table 2).

#### Stability of dialect boundaries and differences between habitats

The results of Mann–Whitney *U* tests of differences in song structure between habitat categories in 2010–11 are shown in Table 3. We found that all variables related to the trill part of

**Table 1. Mean and standard deviation (s.d.) of song variables in closed habitat in 1987, 2000 and 2010–11. *P* values for Kruskal–Wallis analysis of variance (ANOVA) after Bonferroni–Holm correction, with significant comparisons in bold***N* = number of individual songs analysed. For key to song variables, see ‘Methods’ and Fig. 1

	1987 ( <i>n</i> = 137 songs)		2000 ( <i>n</i> = 97 songs)		2010–11 ( <i>n</i> = 126 songs)		<i>P</i>
	Mean	s.d.	Mean	s.d.	Mean	s.d.	
SongD (ms)	1538	202	1650	193	1715	158	< <b>0.001</b>
ThemeD (ms)	939	132	1004	145	969	135	< <b>0.001</b>
TrillD (ms)	491	180	530	170	617	164	< <b>0.001</b>
SongEmp (kHz)	4.52	0.57	4.48	0.57	4.23	0.42	< <b>0.001</b>
ThemeEmp (kHz)	4.73	0.50	4.56	0.51	4.31	0.39	< <b>0.001</b>
TrillEmp (kHz)	3.95	0.51	4.02	0.76	3.74	0.42	< <b>0.001</b>
SongMax (kHz)	7.79	0.75	7.64	0.67	7.85	0.84	0.425
ThemeMax (kHz)	7.66	0.92	7.50	0.86	7.37	1.21	<b>0.024</b>
TrillMax (kHz)	6.90	0.37	6.87	0.40	7.24	0.39	< <b>0.001</b>
SongMin (kHz)	3.26	0.28	3.09	0.30	2.99	0.20	< <b>0.001</b>
ThemeMin (kHz)	3.34	0.33	3.21	0.36	3.18	0.31	< <b>0.001</b>
TrillMin (kHz)	3.45	0.15	3.26	0.15	3.09	0.11	< <b>0.001</b>
NNotes	6.78	2.19	6.02	1.95	6.63	1.92	<b>0.016</b>
TI (ms)	86	12	109	17	113	20	< <b>0.001</b>
TrillBand (kHz)	3.45	0.38	3.61	0.40	4.15	0.40	< <b>0.001</b>

**Table 2. Mean and standard deviation (s.d.) of song variables according in open habitat in 1987, 2000 and 2010–11. *P* values for Kruskal–Wallis ANOVA after Bonferroni–Holm correction with significant comparisons in bold***N* = number of individual songs analysed. For key to song variables, see ‘Methods’ and Fig. 1

	1987 ( <i>n</i> = 100 songs)		2000 ( <i>n</i> = 56 songs)		2010–11 ( <i>n</i> = 58 songs)		<i>P</i>
	Mean	s.d.	Mean	s.d.	Mean	s.d.	
SongD (ms)	1576	218	1615	187	1640	184	0.070
ThemeD (ms)	957	151	1045	129	995	121	< <b>0.001</b>
TrillD (ms)	516	178	470	161	539	172	0.036
SongEmp (kHz)	4.63	0.49	4.47	0.45	4.20	0.44	< <b>0.001</b>
ThemeEmp (kHz)	4.71	0.50	4.50	0.39	4.35	0.43	< <b>0.001</b>
TrillEmp (kHz)	4.46	0.68	4.42	0.78	3.90	0.41	< <b>0.001</b>
SongMax (kHz)	7.90	0.77	7.66	0.83	7.76	1.17	0.239
ThemeMax (kHz)	7.84	0.85	7.61	0.91	7.49	1.40	0.134
TrillMax (kHz)	6.90	0.31	6.66	0.34	6.88	0.50	< <b>0.001</b>
SongMin (kHz)	3.28	0.35	3.14	0.32	3.00	0.26	< <b>0.001</b>
ThemeMin (kHz)	3.32	0.38	3.20	0.33	3.08	0.32	< <b>0.001</b>
TrillMin (kHz)	3.64	0.21	3.45	0.27	3.27	0.19	< <b>0.001</b>
NNotes	8.87	3.16	7.43	2.19	7.86	2.38	<b>0.006</b>
TI (ms)	67.30	11.24	73.29	8.65	80	12	< <b>0.001</b>
TrillBand (kHz)	3.26	0.37	3.21	0.48	3.61	0.55	< <b>0.001</b>

the song differed between habitats: trills from the closed habitat were longer (TrillD), with fewer notes (NNotes) but with longer inter-note interval (TI) compared with trills from open habitat. Trills from closed habitat also had higher maximum frequency (TrillMax), lower minimum frequency (TrillMin) and broader bandwidth (TrillBand) than those from the open habitat. The pattern of variation in the duration of the trill interval (TI) was clinal rather than discontinuous, as was observed in the previous studies (Fig. 2). Total duration of the song (SongD) also varied between habitats but because there were no significant differences in duration of the theme (ThemeD), this difference is explained by the difference in duration of the trill (TrillD) described above. We found no significant differences between habitats in the other variables measured (Table 3).

We found that songs with higher trill interval (TI) had broader trill bandwidth (TrillBand) and this positive correlation was

significant for the three study periods (Fig. 3). Similar results were obtained for 1987 ( $r=0.31$ ,  $n=237$   $P<0.001$ ) and 2000 ( $r=0.37$ ,  $n=153$ ,  $P<0.001$ ).

#### *Changes within dialects*

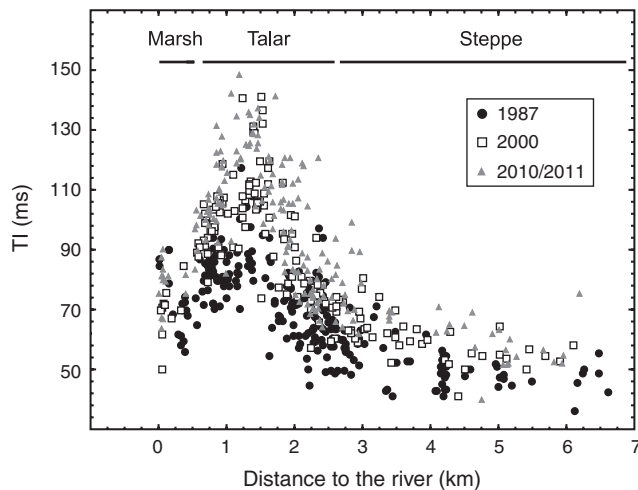
The comparison of our recordings to those from 1987 and 2000 showed that some of the changes reported by Kopuchian *et al.* (2004) had intensified, such as the decrease in minimum frequency of songs (SongMin) and of trill (TrillMin) in both habitats, and the increase in trill interval (TI) in the open habitat (Tables 1 and 2). Other changes, however, had reverted: the decrease in maximum frequency of trill (TrillMax) and number of trill notes (NNotes) in the open habitat and the increase in the duration of the theme (ThemeD) in both habitats (Tables 1, 2). All of these differences were significant (see Tables 4, 5).



**Table 3. Results of Mann–Whitney *U* tests comparing open and closed habitats in 2010–11**

Significant values of *P* marked in bold. For key to song variables, see ‘Methods’ and Fig. 1

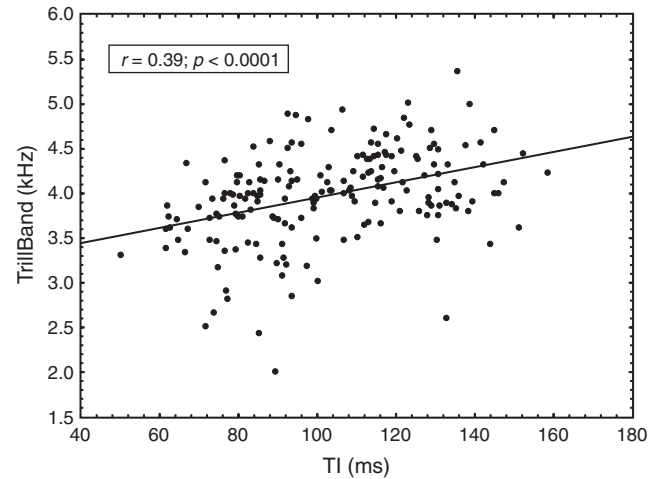
	Closed versus open habitats		
	<i>U</i>	<i>Z</i> <sub>adj</sub>	<i>P</i>
SongD (ms)	2671	2.93	<b>0.003</b>
ThemeD (ms)	3268	-1.15	0.250
TrillD (ms)	2691	2.87	<b>0.004</b>
SongEmp (kHz)	3430	0.67	0.504
ThemeEmp (kHz)	3501	-0.46	0.647
TrillEmp (kHz)	2353	-3.88	<b>&lt;0.001</b>
SongMax (kHz)	3264	1.16	0.245
ThemeMax (kHz)	3533	-0.36	0.717
TrillMax (kHz)	2103	4.62	<b>&lt;0.001</b>
SongMin (kHz)	3552	-0.30	0.761
ThemeMin (kHz)	2934	2.15	0.032
TrillMin (kHz)	1302	-7.01	<b>&lt;0.001</b>
NNotes	2477	-3.56	<b>&lt;0.001</b>
TI (ms)	587	9.14	<b>&lt;0.001</b>
TrillBand (kHz)	1412	6.68	<b>&lt;0.001</b>



**Fig. 2.** Scatter plot of trill intervals (TI) versus distance from the Río de la Plata banks for 1987 and 2000 (Kopuchian *et al.* 2004) and 2010–11 (present study). The approximate distribution of habitats is shown.

We also detected variation between 2010–11 and the earlier studies that was not detected between 1987 and 2000: a decrease in the emphasised frequency of the song (SongEmp), of the theme (ThemeEmp) and of the trill (TrillEmp) in both types of habitat (Tables 1 and 2), a decrease in maximum frequency of the theme (ThemeMax) and an increase in duration (TrillD) and maximum frequency of trills (TrillMax) in closed habitat (Table 1) and the decrease of minimum frequency of themes (ThemeMin) in open habitat (Table 2). All these differences were significant (Tables 4, 5).

Lastly, there were some variables that differed between 1987 and 2000 (Kopuchian *et al.* 2004), but did not differ significantly between 2000 and 2010–11: the increase in duration of the song (SongD) and trill interval (TI) and the decrease in



**Fig. 3.** Scatter plot of trill bandwidth (TrillBand) versus trill interval (TI) in 2010–11.

**Table 4. Results of Mann–Whitney *U* tests comparing songs from 2010–11 with those from 1987 and 2000 for closed habitat**

Significant values of *P* marked in bold. For key to song variables, see ‘Methods’ and Fig. 1

	1987 versus 2010–11			2000 versus 2010–11		
	<i>U</i>	<i>Z</i> <sub>adj</sub>	<i>P</i>	<i>U</i>	<i>Z</i> <sub>adj</sub>	<i>P</i>
SongD (ms)	4173	-7.23	<b>&lt;0.001</b>	4950	-2.43	0.015
ThemeD (ms)	7223	-2.29	<b>0.022</b>	5262	1.78	0.075
TrillD (ms)	5296	-5.41	<b>&lt;0.001</b>	4453	-3.47	<b>0.001</b>
SongEmp (kHz)	6154	4.02	<b>&lt;0.001</b>	4371	3.64	<b>&lt;0.001</b>
ThemeEmp (kHz)	4282	7.06	<b>&lt;0.001</b>	4101	4.21	<b>&lt;0.001</b>
TrillEmp (kHz)	4958	5.96	<b>&lt;0.001</b>	5388	1.52	0.130
ThemeMax (kHz)	7007	2.64	<b>0.008</b>	5337	1.62	0.105
TrillMax (kHz)	4238	-7.13	<b>&lt;0.001</b>	2622	-7.31	<b>&lt;0.001</b>
SongMin (kHz)	3156	8.89	<b>&lt;0.001</b>	3987	4.45	<b>&lt;0.001</b>
ThemeMin (kHz)	5625	4.88	<b>&lt;0.001</b>	5515	1.25	0.212
TrillMin (kHz)	544	13.14	<b>&lt;0.001</b>	2148	8.30	<b>&lt;0.001</b>
NNotes	8209	0.69	0.487	5050	-2.26	0.024
TI (ms)	2302	-10.27	<b>&lt;0.001</b>	5158	-2.00	0.046
TrillBand (kHz)	1734	-11.19	<b>&lt;0.001</b>	1874	-8.87	<b>&lt;0.001</b>

minimum frequency of the theme (ThemeMin) in closed habitat (Tables 1 and 4).

**Discussion**

We compared two song dialects of the Rufous-collared Sparrow across three sampling periods – 1987, 2000 and 2010–11 – and from two main habitat types – open and closed habitats. Our study is the first to compare song in the Rufous-collared Sparrow spanning such a long period (24 years) and one of few to compare three time points for changes in vocal dialects. It is also one of only a few studies that have examined such a small geographical scale with a high sampling density (Tubaro *et al.* 1993; Lijtmaer and Tubaro 2007; Leader *et al.* 2008; Luther and Baptista 2010). Most studies of temporal variation in vocalisations usually compare vocalisations over consecutive years or seasons, or compare two sampling times several years apart (Harbison *et al.* 1999; Goodale and Podos 2010; Baker and Gammon 2006; Azar *et al.* 2014). The first type of comparison, over consecutive years,

**Table 5. Results of Mann–Whitney *U* tests comparing songs from 2010–11 with those from 1987 and 2000 for open habitats**Significant values of *P* marked in bold. For key to song variables, see ‘Methods’ and Fig. 1

	1987 versus 2010–11			2000 versus 2010–11		
	<i>U</i>	<i>Z</i> <sub>adj</sub>	<i>P</i>	<i>U</i>	<i>Z</i> <sub>adj</sub>	<i>P</i>
ThemeD (ms)	2439	−1.66	0.096	1239	2.18	0.029
SongEmp (kHz)	1511	5.01	< <b>0.001</b>	1114	2.89	<b>0.004</b>
ThemeEmp (kHz)	1676	4.42	< <b>0.001</b>	1281	1.94	0.052
TrillEmp (kHz)	1271	5.88	< <b>0.001</b>	1094	3.00	<b>0.003</b>
TrillMax (kHz)	2791	−0.39	0.694	1050	−3.25	<b>0.001</b>
SongMin (kHz)	1372	5.52	< <b>0.001</b>	998	3.55	< <b>0.001</b>
ThemeMin (kHz)	1693	4.36	< <b>0.001</b>	1225	2.26	0.024
TrillMin (kHz)	430	8.93	< <b>0.001</b>	670	5.41	< <b>0.001</b>
NNotes	2362	1.96	0.050	1449	−1.00	0.316
TI (ms)	1226	−6.04	< <b>0.001</b>	1078	−3.09	<b>0.002</b>
TrillBand (kHz)	1602	−4.68	< <b>0.001</b>	836	−4.47	< <b>0.001</b>

usually focuses on bird species where males modify their songs in adulthood (Catchpole and Slater 2008). When song is fixed in adulthood, the rate of change is slower and studies that extend over long periods are needed (Catchpole and Slater 2008). However, studies that extend over longer periods and compare more than two points in time are scarce (see, for example, Derryberry 2011).

#### *Stability of dialect boundaries and differences between habitats*

Our results show that the dialect boundaries of the Rufous-collared Sparrow, in terms of habitat, have remained stable over a period of 24 years: songs from closed habitat (talar woodland) had longer trill intervals than those from the open habitats (marshland and steppe grassland). Trills from the closed habitat also had lower minimum and higher maximum frequencies and thus a wider bandwidth.

Derryberry (2011) found similar results in the congeneric White-crowned Sparrow (*Zonotrichia leucophrys*) for time-spans almost the same as in the present study (12 and 24 years). In White-crowned Sparrows, dialects sung over large areas tend to be stable (over periods of 18–30 years; Trainer 1983; Baker and Thompson 1985; Chilton and Lein 1996; Harbison *et al.* 1999), whereas dialects sung in small areas, as in our study, have shorter lifespans (Chilton and Lein 1996; Harbison *et al.* 1999). However, the distribution of White-crowned Sparrow dialects appears to be a consequence of population changes, such as local extinctions and colonisation of empty patches of habitat (Baker 1975) and not to coincide with changes in habitat as in the Rufous-collared Sparrow. Factors determining the formation and maintenance of dialects vary greatly between these species. Rufous-collared Sparrow dialects are associated with habitat structure in a way that conforms to expectations from acoustical theory (Brown and Handford 1996, 2000). Long-term stability of Rufous-collared Sparrow dialects had been inferred in a previous analysis at a macrogeographical scale (Handford 1988). Because our study site is included in a natural reserve and we detected no significant changes in habitat structure or distribution, such stability was to be expected, even at the small geographical scale of our study.

Previous studies have shown that there are no genetic discontinuities within Argentinean populations of Rufous-collared Sparrows and that phylogeographic structure does not explain patterns of vocal structure (Lougheed *et al.* 2013; Campagna *et al.* 2014). The song-elements that define the dialects are learned by imitation (Egli 1971; Tubaro *et al.* 1997). Our results, taken together with this previous evidence, are consistent with the view that the maintenance of dialects is not a genetic phenomenon but is most likely the consequence of processes such as cultural selection (Crozier 2010). Habitat structure would have acted as a selection factor to maintain dialect boundaries.

The positive correlation between trill interval and frequency bandwidth we found is consistent with that described for other populations of this species (Handford and Lougheed 1991). In our study, the difference in bandwidth is explained in part by differences in minimum frequencies. This pattern of association is not easily predicted by acoustical theory because as long as the singing bird is at least 1 m above the ground, lower frequencies should propagate better than higher ones in all habitats (Wiley and Richards 1982). However, it has been argued elsewhere that, owing to physiological constraints, the shorter the separation between trill notes, the narrower the bandwidth that can be produced (Podos 1997; Podos *et al.* 2004b), as we found. A change of frequency as required by a trill implies a change in the tension of the syrinx tissue, which is the main source of the fundamental frequency of a vocalisation (Mindlin and Laje 2005). Also, it requires a change in bill-gape to change the total length of the vocal tract and track the fundamental frequency produced by the syrinx (Nowicki *et al.* 1992; Podos 1997, 2001). But there is a limit to how fast a bird can change the syrinx tension and bill-gape, determined mainly by how fast the muscles can contract. This constraint imposes a trade-off between rate of notes and frequency bandwidth, which could explain the spectral differences between habitats we found: a bird producing trill notes more separately in time could produce lower minimum frequencies, higher maximum frequencies, or both. Thus, this difference between habitats may not represent an adaptive advantage but may just be a by-product of the temporal characteristics of the trill that were selected in each habitat. This is an interesting example of how a constraint imposed by a vocal mechanism, which is often associated with the idea of stasis in song evolution (Podos *et al.* 2004a), might actually be partly responsible for intraspecific variation in songs among close populations.

#### *Changes within dialects*

Kopuchian *et al.* (2004) detected changes in song in Rufous-collared Sparrows after 13 years in the trill, which defines dialects, and the rest of the song. We found that some of those differences have intensified after a further 10 years, some reverted, and some did not change from 2000 to 2010.

We found a general pattern of decreasing song frequencies, a pattern also suggested by the results of Kopuchian *et al.* (2004). Larger birds are capable of producing songs with lower frequencies (Wallschläger 1980; Ryan and Brenowitz 1985) and lower frequencies transmit better in any kind of habitat (Morton 1975). Morphology of the bill also has an important effect on song frequencies, because the length of the vocal tract can affect the frequencies produced (Palacios and Tubaro 2000). An increase

in body size as well as length of the bill could explain our results. Unfortunately, we could not test this hypothesis since there were no study skins of Rufous-collared Sparrows from our study site captured before 2000, but there is evidence from a closely related species, the White-crowned Sparrow, that bill-size can change in a comparable period (Derryberry 2009). Thus, it is necessary to further explore the hypothesis of variation in learned songs as a consequence of a constraint imposed by the morphology of a bird that has changed over time.

Variation in temporal variables, particularly in intervals between notes, is often related to habitat structure (Morton 1975; Ryan and Brenowitz 1985; Wiley 1991; Tubaro and Lijtmaer 2006). Given that Rufous-collared Sparrow dialects are associated with habitat structure, changes in such dialects could occur after modification of acoustical properties of the environment. We did not detect significant changes in habitat structure or distribution that could explain the changes between seasons we observed. However, our study was not designed to measure habitat variables and the existence of subtle changes in the characteristics of the habitats cannot be ruled out. Also, as stated above, bill morphology can affect the temporal structure of songs (Podos 2001) and the possibility of changes in songs associated with change in bill morphology should also be considered in future studies.

It is likely that some of the observed variation in song structure over the overall study period is a result of neutral change. Errors in the learning process can generate diversification in song structure. As non-deleterious mutations can be fixed by genetic drift, song differences arising from learning errors can also be transmitted in a population and become fixed if they are neutral in relation to the long-range communication properties of the song. It has been shown that changes in a learned trait proceed more rapidly in smaller populations at microgeographical scales (Harbison *et al.* 1999), the scale at which our study was conducted. As stated above, reversal in some changes that had been detected in earlier studies in the absence of factors that could explain such changes indicates neutral variation as the most probable explanation for the changes.

The role of methodological or technical problems in affecting the detection of changes in song, such as differences in the criterion for choosing or measuring songs, or the different devices used (studies in 1987 and 2000 used magnetic recording tape whereas our work recorded digitally) needs to be considered. However, our results suggest that the differences we observed were real and not methodological artefacts. Not all temporal or frequency variables changed in a systematic way between seasons, which would be the case if methodological issues were biasing the results.

## Conclusions

We found that the differences between Rufous-collared Sparrow dialects from open and closed habitats remained after a period of 24 years, maintaining the boundaries of the dialects across time in the study area. Habitat structure, which appeared not to change greatly over the 24 years considered in this study, appears to be the main factor maintaining the spatial distribution of dialects. When considered separately, each dialect has significant differences that persisted across time, some of which were already detected by

Kopuchian *et al.* (2004), whereas other differences showed reversal across the study period. Our results are, therefore, consistent with the idea that some changes in song were a result of neutral variation.

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## References

- Azar, J. F., Bell, B. D., and Borowiec, M. (2014). Temporal change of the song of a local population of the Grey Warbler (*Gerygone igata*): has its song changed over time? *Emu* **114**, 80–85.
- Baker, M. C. (1975). Song dialects and genetic differences in White-crowned Sparrows (*Zonotrichia leucophrys*). *Evolution* **29**, 226–241. doi:10.2307/2407210
- Baker, M. C., and Gammon, D. E. (2006). Persistence and change of vocal signals in natural populations of chickadees: annual sampling of the gargle call over eight seasons. *Behaviour* **143**, 1473–1509.
- Baker, M. C., and Thompson, D. B. (1985). Song dialects of White-crowned Sparrows: historical processes inferred from patterns of geographic variation. *Condor* **87**, 127–141. doi:10.2307/1367144
- Brown, T. J., and Handford, P. (1996). Acoustical signal amplitude patterns: a computer simulation investigation of the acoustic adaptation hypothesis. *Condor* **98**, 608–623. doi:10.2307/1369573
- Brown, T. J., and Handford, P. (2000). Sound design for vocalizations: quality in the woods, consistency in the fields. *Condor* **102**, 81–92. doi:10.1650/0010-5422(2000)102[0081:SDFVQI]2.0.CO;2
- Cabrera, A. L. (1949). Las comunidades vegetales de los alrededores de La Plata. *Lilloa* **20**, 269–347.
- Cabrera, A. L. (1976). Regiones fitogeográficas Argentinas. In 'Enciclopedia Argentina de Agricultura y Jardinería, Volumen 2. Tomo 1. 2nd Edition'. (Ed. M. Dimitri) (Editorial Acme S.A.C.I.: Buenos Aires, Argentina.)
- Campagna, L., Kopuchian, C., Tubaro, P. L., and Loughheed, S. C. (2014). Secondary contact followed by gene flow between divergent mitochondrial lineages of a widespread Neotropical songbird (*Zonotrichia capensis*). *Biological Journal of the Linnean Society* **111**, 863–868. doi:10.1111/bj.12272
- Catchpole, C. K., and Slater, P. J. B. (2008). 'Bird Song. Biological Themes and Variations.' (Cambridge University Press: Cambridge, UK.)
- Chilton, G., and Lein, M. R. (1996). Long-term changes in songs and song dialect boundaries of Puget Sound White-crowned Sparrows. *Condor* **98**, 567–580. doi:10.2307/1369569
- Crozier, G. K. D. (2010). A formal investigation of Cultural Selection Theory: acoustic adaptation in bird song. *Biology & Philosophy* **25**, 781–801. doi:10.1007/s10539-010-9194-6
- Derryberry, E. P. (2009). Ecology shapes birdsong evolution: variation in morphology and habitat explains variation in White-crowned Sparrow song. *American Naturalist* **174**, 24–33.
- Derryberry, E. P. (2011). Male response to historical and geographical variation in bird song. *Biology Letters* **7**, 57–59. doi:10.1098/rsbl.2010.0519
- Egli, W. (1971). Investigaciones sobre el canto de *Zonotrichia capensis chilensis* (Meyen) (Aves, Passeriformes). *Boletín del Museo Nacional de Historia Natural de Chile* **32**, 173–190.
- Goodale, E., and Podos, J. (2010). Persistence of song types in Darwin's finches, *Geospiza fortis*, over four decades. *Biology Letters* **6**, 589–592. doi:10.1098/rsbl.2010.0165



- Handford, P. (1980). Return rates among highland rufous-collared sparrows. *Journal of Field Ornithology* **5**, 176–177.
- Handford, P. (1981). Vegetational correlates of variation in the song of *Zonotrichia capensis*. *Behavioral Ecology and Sociobiology* **8**, 203–206. doi:10.1007/BF00299831
- Handford, P. (1988). Trill rate dialects in the Rufous-collared Sparrow, *Zonotrichia capensis*, in northwestern Argentina. *Canadian Journal of Zoology* **66**, 2658–2670. doi:10.1139/z88-391
- Handford, P., and Loughheed, S. (1991). Variation in duration and frequency characters in the song of the Rufous-collared Sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects and body size. *Condor* **93**, 644–658. doi:10.2307/1368196
- Handford, P., and Nottebohm, F. (1976). Allozymic and morphological variation in population samples of Rufous-collared Sparrow, *Zonotrichia capensis*, in relation to vocal dialects. *Evolution* **30**, 802–817. doi:10.2307/2407819
- Hansen, P. (1979). Vocal learning: its role in adapting sound structures to long-distance propagation, and a hypothesis on its evolution. *Animal Behaviour* **27**, 1270–1271. doi:10.1016/0003-3472(79)90073-3
- Harbison, H., Nelson, D. A., and Hahn, T. P. (1999). Long-term persistence of song dialects in the mountain White-crowned Sparrow. *Condor* **101**, 133–148. doi:10.2307/1370454
- Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**, 65–70.
- King, J. R. (1972). Variation in the song of the Rufous-collared Sparrow, *Zonotrichia capensis*, in northwestern Argentina. *Zeitschrift für Tierpsychologie* **30**, 344–373. doi:10.1111/j.1439-0310.1972.tb00863.x
- Kopuchian, C., Lijtmaer, D. A., Tubaro, P. L., and Handford, P. (2004). Temporal stability and change in a microgeographical pattern of song variation in the Rufous-collared Sparrow. *Animal Behaviour* **68**, 551–559. doi:10.1016/j.anbehav.2003.10.025
- Leader, N., Geffen, E., Mokady, O., and Yom-Tov, Y. (2008). Song dialects do not restrict gene flow in an urban population of the orange-tufted sunbird, *Nectarinia osea*. *Behavioral Ecology and Sociobiology* **62**, 1299–1305. doi:10.1007/s00265-008-0558-8
- Lijtmaer, D. A., and Tubaro, P. L. (2007). A reversed pattern of association between song dialects and habitat in the Rufous-collared Sparrow. *Condor* **109**, 658–667. doi:10.1650/8176.1
- Loughheed, S. C., Campagna, L., Dávila, J. A., Tubaro, P. L., Lijtmaer, D. A., and Handford, P. (2013). Continental phylogeography of an ecologically and morphologically diverse Neotropical songbird, *Zonotrichia capensis*. *BMC Evolutionary Biology* **13**, 58. doi:10.1186/1471-2148-13-58
- Luther, D., and Baptista, L. (2010). Urban noise and the cultural evolution of bird songs. *Proceedings of the Royal Society B: Biological Sciences* **277**, 469–473. doi:10.1098/rspb.2009.1571
- Lynch, A. (1996). The population memetics of birdsong. In 'Ecology and Evolution of Acoustic Communication in Birds'. (Eds D. E. Kroodsma and E. H. Miller.) pp. 181–197. (Cornell University Press: Ithaca, NY.)
- Lynch, A., Plunkett, G. M., Baker, A. J., and Jenkins, P. F. (1989). A model of cultural evolution of chaffinch song derived with the meme concept. *American Naturalist* **133**, 634–653. Available at <http://www.jstor.org/stable/2462072> [Verified 7 July 2015].
- Miller, A. H., and Miller, V. D. (1968). The behavioural ecology and breeding biology of the Andean sparrow, *Zonotrichia capensis*. *Caldasia* **10**, 83–154. Available at <http://www.jstor.org/stable/23641155> [Verified 7 July 2015].
- Mindlin, G. B., and Laje, R. (2005). 'The Physics of Birdsong.' (Springer: Berlin.)
- Morton, E. S. (1975). Ecological sources of selection on avian sounds. *American Naturalist* **109**, 17–34. doi:10.1086/282971
- Mundinger, P. C. (1983). Microgeographic and macrogeographic variation in the acquired vocalizations of birds. In 'Acoustic Communication in Birds. Vol. 2: Song Learning and Its Consequences'. (Ed. D. E. Kroodsma) pp. 147–208. (Academic Press: New York.)
- Nottebohm, F. (1969). The song of the Chingolo, *Zonotrichia capensis*, in Argentina: description and evaluation of a system of dialects. *Condor* **71**, 299–315. doi:10.2307/1366306
- Nottebohm, F. (1975). Continental patterns of song variability in *Zonotrichia capensis*: some possible ecological correlates. *American Naturalist* **109**, 605–624. doi:10.1086/283033
- Nowicki, S., Westneat, M., and Hoese, W. (1992). Birdsong: motor function and the evolution of communication. *Seminars in Neuroscience* **4**, 385–390. doi:10.1016/1044-5765(92)90046-5
- Palacios, M. G., and Tubaro, P. L. (2000). Does beak size affect acoustic frequencies in woodcreepers? *Condor* **102**, 553–560. doi:10.1650/0010-5422(2000)102[0553:DBSAAF]2.0.CO;2
- Parodi, L. R. (1940). La distribución geográfica de los talaes de la Provincia de Buenos Aires. *Darwiniana* **4**, 33–56.
- Podos, J. (1997). A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). *Evolution* **51**, 537–551. doi:10.2307/2411126
- Podos, J. (2001). Correlated evolution of morphology and vocal signal structure in Darwin's Finches. *Nature* **409**, 185–188. doi:10.1038/35051570
- Podos, J., and Warren, P. S. (2007). The evolution of geographic variation in birdsong. *Advances in the Study of Behavior* **37**, 403–458.
- Podos, J., Huber, S. K., and Taft, B. (2004a). Bird song: the interface of evolution and mechanism. *Annual Review of Ecology, Evolution and Systematics* **35**, 55–87. doi:10.1146/annurev.ecolsys.35.021103.105719
- Podos, J., Southall, J. A., and Rossi-Santos, M. R. (2004b). Vocal mechanics in Darwin's Finches: correlation of beak gape and song frequency. *Journal of Experimental Biology* **207**, 607–619. doi:10.1242/jeb.00770
- Richards, D. G., and Wiley, R. H. (1980). Reverberations and amplitude fluctuations in the propagation of sound in a forest: implications for animal communication. *American Naturalist* **115**, 381–399. Available at <http://www.unc.edu/home/rhwiley/pdfs/ReverberationsAmplitudes.pdf> [Verified 7 July 2015].
- Ryan, M. J., and Brenowitz, E. A. (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. *American Naturalist* **126**, 87–100. doi:10.1086/284398
- Trainer, J. M. (1983). Changes in song dialect distributions and microgeographic variation in song of White-crowned sparrows (*Zonotrichia leucophrys nuttalli*). *Auk* **100**, 568–582.
- Tubaro, P. L., and Lijtmaer, D. A. (2006). Environmental correlates of song structure in forest grosbeaks and saltators. *Condor* **108**, 120–129. doi:10.1650/0010-5422(2006)108[0120:ECOSI]2.0.CO;2
- Tubaro, P. L., Segura, E. T., and Handford, P. (1993). Geographic variation in the song of the Rufous-collared Sparrow in eastern Argentina. *Condor* **95**, 588–595. doi:10.2307/1369603
- Tubaro, P. L., Handford, P., and Segura, E. T. (1997). Song learning in the Rufous-collared Sparrow (*Zonotrichia capensis*). *Hornero* **14**, 204–210.
- Wallschläger, D. (1980). Correlation of song frequency and body weight in passerine birds. *Experientia* **36**, 412. doi:10.1007/BF01975119
- Wiley, R. H. (1991). Associations of song properties with habitats for territorial oscine birds of eastern North America. *American Naturalist* **138**, 973–993. doi:10.1086/285263
- Wiley, R. H., and Richards, D. G. (1978). Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. *Behavioral Ecology and Sociobiology* **3**, 69–94. Available at <http://www.jstor.org/stable/4599157> [Verified 7 July 2015].
- Wiley, R. H., and Richards, D. G. (1982). Adaptations for acoustic communication in birds: sound transmission and signal detection. In 'Acoustic Communication in Birds. Vol. 1: Production, Perception, and Design Features of Sound'. (Eds D. E. Kroodsma and E. H. Miller.) pp. 132–181. (Academic Press: New York.)