

Does vocal learning accelerate acoustic diversification? Evolution of contact calls in Neotropical parrots

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Abstract

Learning has been traditionally thought to accelerate the evolutionary change of behavioural traits. We evaluated the evolutionary rate of learned vocalizations and the interplay of morphology and ecology in the evolution of these signals. We examined contact calls of 51 species of Neotropical parrots from the tribe Arini. Parrots are ideal subjects due to their wide range of body sizes and habitats, and their open-ended vocal learning that allows them to modify their calls throughout life. We estimated the evolutionary rate of acoustic parameters of parrot contact calls and compared them to those of morphological traits and habitat. We also evaluated the effect of body mass, bill length, vegetation density and species interactions on acoustic parameters of contact calls while controlling for phylogeny. Evolutionary rates of acoustic parameters did not differ from those of our predictor variables except for spectral entropy, which had a significantly slower rate of evolution. We found support for correlated evolution of call duration, and fundamental and peak frequencies with body mass, and of fundamental frequency with bill length. The degree of sympatry between species did not have a significant effect on acoustic parameters. Our results suggest that parrot contact calls, which are learned acoustic signals, show evolutionary rates similar to those of morphological traits. This is the first study to our knowledge to provide evidence that change through cultural evolution does not necessarily accelerate the evolutionary rate of traits acquired through life-long vocal learning.

Introduction

Learned behaviours are often hypothesized to be more labile than innate behaviours or morphological traits due to their potential for rapid change through cultural evolution (West-Eberhard, 1983; Blomberg *et al.*, 2003; Lachlan & Servedio, 2004). As a consequence of this potential lability, it is sometimes assumed that learned behaviours, such as avian vocalizations with a strong vocal learning component (e.g. calls and song in oscine songbirds, parrots, hummingbirds), diverge too rapidly to permit effective comparative studies (Price & Lanyon, 2002; Seddon, 2005; Tobias *et al.*, 2010). Such rapid

diversification may be particularly strong in species with rapid sexual selection (Price & Lanyon, 2002; Cardoso *et al.*, 2012; Seddon *et al.*, 2013), or in open-ended learning taxa such as parrots (Bradbury, 2003; Dahlin *et al.*, 2013) and some hummingbirds (Araya-Salas & Wright, 2013) that can alter their vocalizations throughout their lifespan. Vocal dialects, which represent substantial variation in learned vocalizations within the same species, are an example of diversification of a learned behaviour (Baker, 1975; Jenkins, 1978; Rothstein & Fleischer, 1987; Wright, 1996). Although learning clearly has the potential to generate signal diversification and therefore may play an important role in speciation (Danchin *et al.*, 2011; Wilkins *et al.*, 2013; Gill, 2014), there is no empirical evidence that the differences seen across populations due to cultural evolution have repercussions above the level of species by accelerating the pace of evolutionary change. Furthermore, it remains unclear whether the rate of

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diversification of learned traits is limited by morphological and ecological constraints.

Acoustic signals are a particularly well-suited system to study the interplay of morphological and ecological factors on the evolution of learned behaviours because they can be dissected into multiple components that may be influenced by different selective pressures. A growing body of evidence suggests that acoustic signals are shaped by both morphological and ecological factors in insects (Couldridge & van Staaden, 2004), anurans (Zimmerman, 1983; Kime *et al.*, 2000; Bosch & De la Riva, 2004), mammals (Brown *et al.*, 1995; Mitani & Stuht, 1998; Peters & Peters, 2010) and birds. Comparative studies in avian taxa have shown that morphological traits involved in signal production, such as body size (Ryan & Brenowitz, 1985; Podos, 2001; Seddon, 2005; Price, 2008; Martin *et al.*, 2011), and bill size (Podos, 2001; Seddon, 2005; Derryberry *et al.*, 2012), play a critical role in shaping acoustic signals in birds. The selective pressure for enhanced transmission in the habitat where acoustic signals are produced and received also may shape their structure, as predicted by the 'acoustic adaption' (Morton, 1975) or 'sensory drive' hypotheses (Endler, 1992; Tobias *et al.*, 2010). Support for these ideas is provided both by within species (Derryberry, 2009; Ey & Fischer, 2009) and comparative studies (Seddon, 2005; Tobias *et al.*, 2010; Weir *et al.*, 2012). Another ecological factor that is thought to play a role in the divergence in avian vocalizations is character displacement resulting from species interactions (Seddon, 2005; Tobias *et al.*, 2010). The main prediction of this hypothesis is that signals of closely related species will be more divergent in sympatry than in allopatry, as has been documented in vocalizations of some avian taxa (Seddon, 2005; Kirschel *et al.*, 2009; Grant & Grant, 2010).

The parrots (Order Psittaciformes) are a promising group in which to investigate the pace of evolutionary change of learned behaviours such as life-long acquired vocalizations. Contact calls, which are ubiquitous in parrots, are used for long distance communication and are easily identified as homologous traits. These calls have a strong learning component (Wright, 1996; Wan-ker & Fischer, 2001; Bond & Diamond, 2005; Berg *et al.*, 2011; Ribot *et al.*, 2012) and can be modified in the course of a lifetime (Hile *et al.*, 2000; Manabe *et al.*, 2008; Salinas-Melgoza & Wright, 2012; Dahlin *et al.*, 2013). Parrot contact calls are used by both sexes and all age groups to mediate social interactions, such as location of group members, establishing contact with mates and coordinating foraging activities (Cortopassi & Bradbury, 2006). Therefore, these vocalizations are unlikely to be under strong sexual selection, making them an ideal system to study the interplay of morphological and ecological factors on evolutionary patterns of learned acoustic signals, in the absence of the effects of sexual selection on signal diversification.

Here, we examine the evolution of learned contact calls in a monophyletic group of Neotropical parrots of the tribe Arini (Family Psittacidae, *sensu* Joseph *et al.*, 2012). A comprehensive phylogeny of this tribe is available (Schirtzinger, 2011), and species in the group show a wide range of sizes and occupy a variety of habitats in Central and South America and the Caribbean (Forshaw, 2006). We evaluated the hypothesis that life-long learned vocalizations exhibit a higher pace of evolutionary change. We also evaluated whether lower rates of evolution are found in acoustic parameters that co-evolved to morphological/ecological factors. We estimated and compared evolutionary rates of both acoustic parameters and measures of morphology and habitat. Then we investigated the potential factors that constrain the diversification of these vocalizations by testing correlated evolution of acoustic parameters and morphological and habitat predictors using phylogenetically generalized least squares (PGLS). We also examined the effect of range overlap using a distance matrix approach that controlled for genetic distance between pairs of species.

Materials and methods

Sound recordings

We obtained 285 recordings from 51 species of the tribe Arini *sensu* Schodde *et al.* (2013) from bioacoustics archives (Table S1). Three species of the 55 species present in the phylogeny were not included in the call database due the small numbers of recordings available (<3 recordings for each). Another species was excluded due to lack of habitat data. We sampled an average 5.58 recordings (min: 3, max: 10) per species (Table S1).

We extracted one call per recording to ensure each recording represented a single individual. The sex of the individual was unknown for most of the recordings. To guide our selection of contact calls from field recordings, we chose one representative call per species, from the CD 'Voices of New World Parrots' (Whitney *et al.*, 2002) as the reference contact call for each species during further sampling from other databases (Table S1). This source was used as the reference due to the certainty of species and call type identification in these recordings, as well as their high audio quality. Calls obtained from other collections were compared to these reference calls and only those similar to their respective reference, based on visual inspection of the spectrograms, were included in the analyses. All the files obtained in 'mp3' format were converted into 'wav' format (44.1 KHz, 16 bits).

Measurement of acoustic parameters

We used the R packages SEEWAVE (Sueur *et al.*, 2008) and warbleR (Araya-Salas *et al.*, 2015) to measure

acoustic parameters. We measured the following: duration, mean frequency (average frequency of the spectrum), skewness (asymmetry of the spectrum), kurtosis (peakedness of the spectrum), spectral entropy (energy distribution; pure tone ~ 0 ; noisy ~ 1), fundamental frequency (average of 10 measures of the fundamental frequency equally spaced throughout the call), dominant frequency (average of ten equally spaced measures of the dominant frequencies throughout the call), peak frequency (frequency with the highest amplitude), frequency range (difference between the lowest and the highest fundamental frequency in a call), modulation index (accumulated absolute difference between adjacent measurements of fundamental frequencies divided by the frequency range) and peak time (ratio of the time at which the highest amplitude is reached to the duration of the call). Note that peak frequency is equivalent to the 'dominant frequency' used in some other studies and to the 'maximum frequency' measurement in the software RAVEN 1.3 and 1.4 (Cornell Lab of Ornithology, Ithaca, NY, USA).

Testing the effects of audio compression

Calls available in the online repository Xeno-canto are typically uploaded in a file format using audio compression algorithms (e.g. 'mp3'), whereas the other calls used were saved in formats without compression (e.g. 'aiff' or 'wav'). We assessed the effect of audio file compression by comparing the acoustic parameters from sound files that were originally in uncompressed format to their compressed version. Sound files that were obtained in 'wav' format were compressed to 'mp3' format at 64 kbps (only six of the 224 originally compressed recordings had a sampling rate < 64 kbps) and then converted back to 'wav' (44 kHz, 16 bits) in the same fashion as recordings originally obtained in compressed format. Then we compared individual acoustic parameters for compressed and uncompressed files with a paired *t*-test. Parameters significantly affected by compression were excluded from further analyses as detailed below. In addition, we identified collinearity among the remaining parameters ($r > 0.7$; Dormann *et al.*, 2012) using Pearson product-moment correlation test and excluded collinear parameters.

Morphological and habitat data

The body mass for all the species was obtained from Dunning (2008) and Forshaw (2006). For 15 species for which body mass was not available, we extrapolated a mass from congeners of similar body length for which body mass data were available. Bill sizes were obtained from Forshaw (2006).

We used the enhanced vegetation index (hereafter 'vegetation index'), which represents habitat characteristics as biomass and leaf area index, as a proxy for

habitat structure. We obtained vegetation indices from global climate modelling grids (MOD13C2 MODIS layers) taken monthly at a 5600 m (0.05 degrees) resolution (available at https://lpdaac.usgs.gov/dataset_discovery/modis/modis_products). A single vegetation index layer was calculated by averaging five randomly selected years (60 months) from the available period (2001–2012). Unsuitable habitats (e.g. cities, water bodies) were excluded to ensure the index reflected the structure of the natural habitat of the species. From the average vegetation index layer, we removed the grid cells categorized as 'artificial surfaces', 'bare areas', 'inland water bodies' and 'irrigated croplands' based on the 2009 ESA global land cover map (Bontemps *et al.*, 2011). Finally, we obtained an average vegetation index for each species by extracting the vegetation index across the entire distribution range. Distribution maps were obtained from <http://www.birdlife.org> (BirdLife International and NatureServe, 2012). These maps reflect range updates from the American Ornithological Union (Chesser *et al.*, 2011) and the South American Checklist Committee through 1 May 2012. We validated this vegetation index by comparing the values for a subset of species that exclusively occupy 'wet forest' against those that only occupy 'dry forest', while accounting for latitude (covariate). Habitats descriptions used for this analysis were obtained from <http://www.birdlife.org>. As expected, we found that species from 'wet forest' presented significantly higher vegetation index values than those from 'dry forest' ($F = 8.04$, d.f. = 2/13, $P = 0.0053$).

Phylogenetic analyses and estimation of evolutionary rate

We used phylogenetic trees for the tribe Arini that were derived as part of a larger investigation of the relationships within the Neotropical parrots (Subfamily Arinae) by Schirtzinger (2011) that is the most comprehensive phylogeny to date for this group. The larger phylogeny sampled 129 parrot species, including representatives of 31 of 32 extant genera and all 55 currently recognized extant species of the tribe Arini. Four gene regions were sampled: 2 mitochondrial coding genes (cytochrome c oxidase I (COI) and nicotinamide adenosine dehydrogenase subunit 2 (ND2) and 2 nuclear introns (tropomyosin intron 5 (Trop) and transforming growth factor beta 2 intron 1 (TGFB2) for a total of 2789 base pairs sequenced. Sequences were aligned with Clustal W and gaps within introns coded with the simple indel coding method. Initial analyses were conducted to determine the most effective partitioning scheme for protein coding genes using partition-specific models determined by MRMODELTEST2.2 (Posada & Crandall, 1998) and then comparing trees recovered in a partitioned Bayes analysis for each separate strategy. Separate tree searches for each partitioning scheme

were conducted in MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003), each with two parallel runs, each with one cold chain and three heated chains run with default parameters for 13 million generations. The resulting trees were compared by calculating the Bayes factor (Kass & Raftery, 1995). This analysis determined that the best partitioning model was the most complex one, which partitioned the two coding regions by coding position, treated the two introns as separate partitions, and included alignment gaps in the introns created by insertions and deletions as the ninth partition. The best fit models for most mitochondrial partitions were GTR + I + G; COI first codon positions were best fit by a SYM + I + G model, whereas COI second codon positions were fit by an F81 model. The two introns were best fit by GTR + R models and the gaps with a restriction data with variable rates model. The best tree from this nine-partition search had a likelihood of $\ln L = -44165.9$, and the 50% majority consensus tree from the 19 500 trees in the posterior distribution had high nodal support in general and for the monophyly of Arini in particular. Its topology was generally congruent with a maximum likelihood analysis of the same data and with previous genus-level phylogenies of parrots that included Neotropical parrots (Schirtzinger, 2011). Trees for subsequent analyses were drawn from the set of 3017 trees with the highest posterior probabilities in the nine-partition Bayes analysis. The subtree for Arini pruned from this tree is shown in Figure S1.

We tested whether the level of phylogenetic signal (λ) in acoustic parameters differed from 0 (no-phylogenetic signal) and 1 (pure Brownian process). Phylogenetic signal measures the similarity of a trait among species that is due to common ancestry (Pagel, 1999). We evaluated correlated evolution between single acoustic parameters and the morphology and habitat structure predictors using phylogenetic generalized least squares (PGLS) comparative method (Freckleton, 2000, 2002) to control for relatedness among species. This method estimates associations between variables while accounting for the phylogenetic signal in the data. We used restricted maximum likelihood estimation with an initial λ value of 0.5 for regression models. For all statistical analyses, both the response variable and the predictors were natural log-transformed. We used four models of trait evolution to select the best fit for regressions: Blomberg's accelerated/decelerated (ACDC) model (Blomberg *et al.*, 2003), Brownian motion (Felsenstein, 1985), Ornstein-Uhlenbeck (OU) process with a single optimum (Hansen, 1997) and Pagel's Lambda model (Pagel, 1999).

We used the biased-corrected version of the Akaike information criteria for small samples as a measure of model fit (AIC_c , Akaike, 1974; Hurvich & Tsai, 1989). Most plausible models were identified as the lowest AIC_c model and any differing by less than 2 units from

this one (Burnham & Anderson, 2002). We used the R packages 'ape' (Paradis *et al.*, 2004) and 'nlme' (Pinheiro *et al.*, 2010) for phylogenetic analyses and 'pgirmess' for model selection (Giraudeau, 2013).

We measured the rate of evolution for both acoustic parameters and ecological/morphological predictors using a recently developed statistical method (Adams, 2013). This method compares the likelihood of a model where each trait evolves at a distinct evolutionary rate to the one of a model where all traits are constrained to evolve at a common evolutionary rate. Pairwise tests were run between all combinations of log-transformed acoustic parameters and predictors. This log-transformation provides a scale-free estimate of evolutionary rates, potentially allowing the comparison of traits that were originally measured in different units (Gingerich, 2009; Adams, 2013), a point that is discussed in further detail below.

All analyses were replicated on different trees to account for phylogenetic uncertainty. Each test was replicated 100 times, each time with a different subtree for Arini that was randomly sampled from the 3017 trees with the highest posterior probability from the Bayes search. The proportion of tests that reached statistical significance ($P < 0.05$ or $\Delta AIC_c < 2$) was used as the associated probability. The calculation of phylogenetic signal, the PGLS analyses, and the estimation of evolutionary rates all require the use of ultrametric trees. We used the correlated model of substitution (Paradis, 2013) to generate ultrametric trees.

Evaluating call divergence by character displacement

We investigated the role of character displacement in the divergence of flight calls by evaluating the relationship between degree of sympatry and the acoustic divergence of flight calls while controlling for phylogenetic relationships. We calculated the proportion of the geographic range in which species pairs are sympatric. For each pair, we calculated two measures of geographic overlap: (i) proportion of species A range within species B range and (ii) proportion of species B range within species A range. The two values were averaged to produce a single proportion of pairwise geographic overlap.

Acoustic dissimilarity was calculated as the species pairwise Euclidean distance derived from a PCA on the acoustic parameters. A matrix of phylogenetic pairwise distances between species derived from our phylogenetic hypothesis was used to account for acoustic similarity due to common ancestry. We applied a partial Mantel test (10 000 permutations) to assess the influence of pairwise geographic overlap in the divergence of acoustic signals, while accounting for phylogenetic distance.

Results

Audio file compression had a significant effect on three acoustic parameters: dominant frequency, frequency range and modulation index ($P < 0.05$ in all cases). These parameters were excluded from further analysis. Skewness and mean frequency were also excluded due to collinearity with other parameters ($r > 0.7$). Values of the acoustic parameters for each species are provided in Table S2.

Most acoustic parameters showed significant phylogenetic signal ($\lambda > 0$); only frequency range and peak frequency did not show phylogenetic signal (Table 1). Likewise, morphological traits and vegetation index had significant phylogenetic signal. On the other hand, only the phylogenetic signal of morphological traits and spectral entropy did not differ significantly from that expected under a Brownian process ($\lambda = 1$).

Three acoustic parameters (duration, peak frequency and fundamental frequency) showed correlated evolution to body mass. Fundamental frequency was also correlated to bill length (Fig. 1). The best regression models are shown in Table 2: in all cases, the Pagel's lambda model of trait evolution was the best fit model of those tested (Table S3). In addition, we conducted generalized least squared regressions accounting for phylogeny among morphological predictors (i.e. body mass and bill length) and the vegetation index. Body mass and bill length were strongly associated (pseudo $R^2 = 0.95$, $P < 0.00001$). Body mass was not significantly correlated to vegetation index (pseudo $R^2 = 0.042$, $P = 0.999$), nor was bill length (pseudo $R^2 = 0.037$, $P = 0.98$).

Evolutionary rates of acoustic parameters did not differ from those of morphological traits and vegetation index, except for spectral entropy, which had an estimated evolutionary rate lower than the other acoustic

parameters and the predictor variables (Fig. 2). See Table S4 for results of pairwise analyses for all traits.

Pairwise acoustic distance was calculated using the first three principal components of the PCA performed on acoustic parameters (Table S2), which explained 75.7% of the variation in the acoustic parameters. A partial Mantel test showed no correlation between species sympatry and acoustic distance while controlling for genetic distance ($r = -0.73$, $P = 0.86$). Acoustic and phylogenetic distances were significantly correlated ($r = 0.28$, $P < 0.0001$), further supporting a strong phylogenetic effect on call evolution in this clade.

Discussion

Our results indicate that learned contact calls in Neotropical parrots contain significant levels of phylogenetic signal. On the other hand, although habitat structure did show a significant degree of phylogenetic signal, it did not show a general pattern of association with acoustic call parameters. Our results further suggest that character displacement is not an important factor on acoustic divergence of Neotropical parrots contact calls, although further analysis taking into account evolutionary time are warranted (e.g. Tobias *et al.*, 2014). Most importantly, our results suggest that life-long learned contact calls might have evolved at a similar rate to morphological traits. To our knowledge, this is the first study to show that traits acquired through life-long learning might evolve at comparable rates to morphological traits.

Phylogenetic signature and evolutionary rate of learned contact calls and other traits

All acoustic parameters, except for frequency range and peak time, exhibit a significant degree of phylogenetic

Table 1 Phylogenetic signal (λ) of acoustic parameters, vegetation index, body mass and bill size. Associated probability (Assoc. P) is indicated as the percentage of tests that reached statistical significance ($P < 0.05$) for $\lambda = 0$ and $\lambda = 1$, respectively. $\lambda = 0$ indicates loss of phylogenetic signal and $\lambda = 1$ indicates that the trait has evolved following a pure Brownian motion in the phylogeny. $0 < \lambda < 1$ indicates partial dependence of the phylogeny.

Acoustic Parameter/ Predictor	λ	$\lambda = 0$			$\lambda = 1$		
		Log-likelihood	Log-likelihood ratio	% tests with $P < 0.05$	Log-likelihood	Log-likelihood ratio	% tests with $P < 0.05$
Duration	0.746	-218.369	28.134	100	-220.309	32.472	100
Peak freq.	0.634	-300.452	14.192	100	-304.477	22.695	100
Kurtosis	0.815	-393.641	13.320	100	-392.710	11.536	100
Spectral Entropy	0.927	-159.421	29.952	100	-148.749	8.145	88
Fund. freq.	0.613	-278.009	16.587	100	-276.023	12.457	97
Freq. range	0.236	-284.538	2.434	0	-291.793	16.951	100
Peak time	0.224	-192.461	3.213	1	-206.475	31.396	100
Vegetation index	0.604	-191.439	7.910	100	-200.168	25.498	100
Body Mass	0.981	-417.934	77.495	100	-381.415	4.820	32
Bill length	0.965	-375.725	28.134	100	-344.004	32.472	11

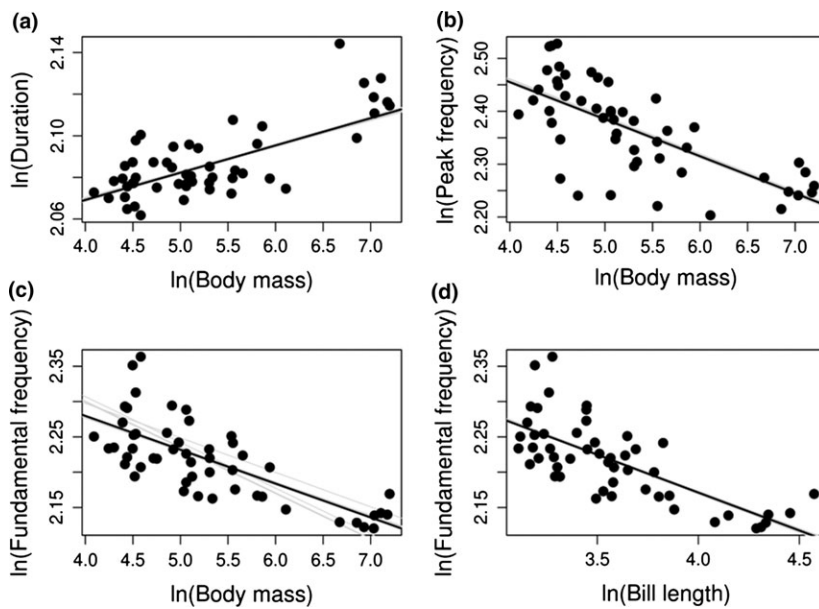


Fig. 1 Associations between log-transformed body mass and bill length (a–d) and log-transformed acoustic parameters. Grey lines indicate best fit lines for different phylogenetic trees. The black line indicates the average fit for the 100 phylogenetic trees.

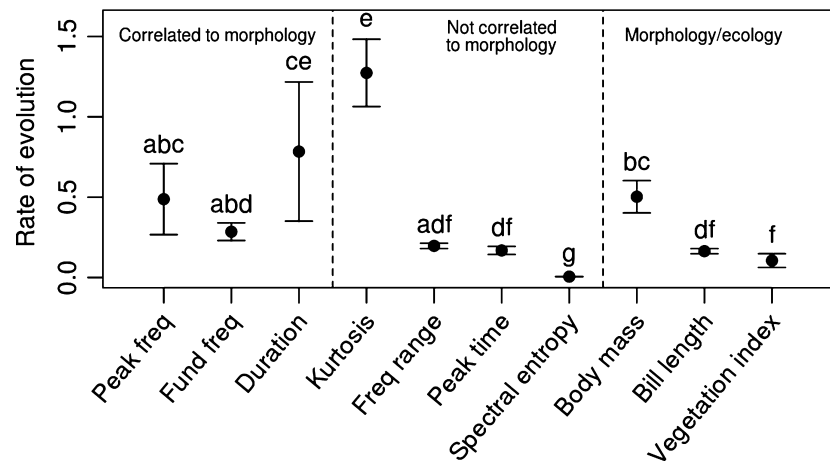
Table 2 Best regression models of acoustic parameters with body mass, bill length and vegetation index ($n = 51$ taxa) controlling for phylogenetic effects. AIC and AIC_c values, AIC and AIC_c differences (Δ), and Akaike weights (w) are shown. The mean slope and intercept for the regression models are reported along with the number of tests in which $\Delta\text{AIC}_c < 2$ and $P < 0.05$.

Acoustic Parameter	Predictor	AIC _c	ΔAIC_c	w (ic)	Tests ($P < 0.05$)	Slope	Intercept	Tests ($\Delta\text{AIC} < 2$)
Duration	Body mass	-282.863	0	0.385	100	0.013	2.017	100
Peak freq.	Body mass	-118.189	0	0.359	100	-0.070	2.736	100
Kurtosis	Body mass	74.730	0	0.643	0	0.017	3.507	100
Spectral Entropy	Body mass	-391.985	0	0.638	0	0.001	2.155	100
Fund. freq.	Body mass	-163.393	0	0.158	100	-0.048	2.470	100
	Bill length	-162.733	0.661	0.110	100	-0.107	2.601	99
Freq. range	Body mass	-124.429	0	0.615	1	-0.019	2.442	100
Peak time	Body mass	-302.100	0	0.823	0	0.002	2.100	100

signal (Table 1). These results indicate that some portion of the variation in the components of the learned contact calls of Neotropical parrots is due to common ancestry; however, there may also be additional variation that cannot be attributed to common ancestry. The results also indicate that some components of these contact calls (e.g. frequency range and peak time) are more labile than others with lambdas that did not differ from zero. The lambdas associated with body mass and bill length were not statistically different than 1, which suggests that these two traits evolved following a pure Brownian motion in the phylogeny. On the other hand, the vegetation index showed a partial independence of the phylogeny ($0 < \lambda < 1$). Taken together, these results suggest that closely related species show similarities in certain acoustic parameters of their contact calls as well as similar morphological traits (body size and bill length) and habitats (vegetation index).

The similarities in evolutionary rates found between acoustic parameters of contact calls and morphological traits (e.g. body mass and bill length) indicate that life-long learned behaviours may not evolve as rapidly as often assumed. Surprisingly, one acoustic trait, spectral entropy, had an evolutionary rate lower than that of body mass and bill length. Although here we use a recently developed statistical tool designed to compare evolutionary rates across multiple traits (Adams, 2013), these comparisons must be interpreted cautiously particularly when comparing traits that were originally measured in different units (e.g. fundamental frequency in KHz vs. body mass in g). Here, we used the approach advocated by several authors (e.g. Gingerich, 2009; Adams, 2013; L. Revell, pers. comm.) of using log-transformed variables for comparing evolutionary rates, because after log-transformation the comparison is based on relative change of scale-free variables.

Fig. 2 Mean evolutionary rates and pairwise comparisons between log-transformed body mass, bill length, vegetation index and acoustic parameters. Confidence intervals are based on standard error of evolutionary rate estimates from different phylogenetic trees. Different letters indicate significantly different evolutionary rates after pairwise comparisons between traits.



However, the biological meaning of the direct comparison of evolutionary rates of traits originally measured in different units is still under debate. Taking this caveat into consideration, overall the evolutionary rates observed across morphological, acoustic and habitat traits lie within a similar range (Fig. 2), which indicates that acoustic signals might be as conserved as morphological traits. We expected to see similar evolutionary rates between morphological traits and acoustic traits that are strongly associated with morphology (e.g. peak and fundamental frequency and duration); however, we did not expect to see these similarities between morphological traits and acoustic parameters unrelated to morphology such as frequency range and spectral entropy. It remains unclear which factors might control the pace of evolution of such acoustic parameters as they did not show a strong association with either morphology or the vegetation index (discussed below).

We can conclude that acoustic parameters of the learned contact calls of Neotropical parrots are highly conserved, which is consistent with the slow evolutionary rate found in acoustic signals of tropical songbirds compared with those in temperate latitudes (Weir & Wheatcroft, 2011). Some authors have suggested that phenotypic diversification promoted by song learning could increase speciation (Baptista *et al.*, 1992; Danchin *et al.*, 2011; Gill, 2014). If learning does not generate signal diversification by accelerating the pace of evolution, then the link between learning and speciation likely would be weak. Further research is necessary to understand the implications of low evolutionary rates of learned vocalizations for diversification rates.

Relationships between morphological predictors and acoustic parameters

We found that body mass is negatively related to peak frequency, and fundamental frequency and positively

related to the duration of the call (Fig. 1). Larger birds may have larger lung capacity and longer vocal tracts than smaller birds and therefore are able to produce longer calls (Bradbury & Vehrencamp, 2011). This relationship has been reported for caciques and oropendolas (Price & Lanyon, 2004), *Phylloscopus* and *Hippolais* warblers (Badyaev & Leaf, 1997), and Asian barbets (Gonzalez Voyer *et al.*, 2013). Larger parrots typically have larger bills, as we demonstrate here; therefore, the positive correlation seen between bill length and fundamental frequency can be explained the same way as the relationship between body mass and fundamental frequency. Larger birds are also likely to have a larger syrinx (the avian sound production organ) than smaller birds. The avian vocal tract, including the bill, is thought to function as an acoustic resonance filter during sound production, with the size of the tract constraining the length of the wavelengths produced (Nowicki, 1987; Podos & Nowicki, 2004; Bradbury & Vehrencamp, 2011). As a consequence, larger parrots, with larger bills, are able to produce lower fundamental frequencies with longer wavelengths than smaller parrots.

We did not observe a significant association between call frequency range and bill length. Podos (2001) found that larger Darwin's finches produce songs with narrower frequency range than smaller finches and several studies have found a general trade-off between frequency range and speed of the repeated notes of trills (Podos, 1997; Podos *et al.*, 2009; Derryberry *et al.*, 2012) which is also largely influenced by bill length (Derryberry *et al.*, 2012). However, emberizids and other avian taxa face trade-offs between frequency bandwidth and trill speed that psittacines do not necessarily face because the latter typically do not produce calls with the rapidly repeated frequency sweeps that characterize the trills of these songbird taxa. Brittan-Powell *et al.* (1997) found no evidence for suprasyringeal control by beak opening of calls in a

small parrot, the budgerigar (*Melopsittacus undulatus*), which is consistent with our findings. These contrasting results illustrate the importance of taking into account differences in vocal production across different avian taxa when predicting the effects of morphology on signal structure.

Relationships between habitat and acoustic parameters

We found no effects of habitat structure on body size or bill length; thus, we can rule out a potential indirect effect of ecology on parrot call evolution through morphological factors. We did not find any significant associations between habitat and acoustic parameters. These results stand in contrast to the negative association between frequency and vegetation density predicted by the acoustic adaptation hypothesis and demonstrated in several bird species (Seddon, 2005; Derryberry, 2009; Ey & Fischer, 2009; Tobias *et al.*, 2010; Weir *et al.*, 2012). This contrast could be explained by interspecific variation in habitat usage. Parrot species often differ in the height at which they fly and broadcast their contact calls relative to the forest canopy. For instance, *Ara* and *Forpus* species that are found in the dense tropical forests of the Manu National Park in Peru typically fly 5 m or more above the canopy (Gilardi & Munn, 1998); these genera contain species with the largest and smallest body sizes, respectively, in the Neotropics. In contrast, species in the genera *Pyrhura* and *Pionopsitta* found at the same site typically fly below the canopy and thus would be more subject to selection from the habitat on acoustic signal form (Gilardi & Munn, 1998). Unfortunately, a lack of comprehensive data on habitat usage by species in Arini precludes a more in-depth analysis at this point.

A further explanation for the general lack of association found between call acoustic parameters and the vegetation index is the use of multiple habitats by many Neotropical parrots. For instance, *Amazona auropalliata* is commonly found in both savannahs and forests and may move between these habitats in the course of a single day (Salinas-Melgoza *et al.*, 2013). Other species, like *Ara ararauna*, moves seasonally between different habitats (Forshaw, 2006). Furthermore, many parrot species studied here exhibit regional variation in habitat use occupy different habitats in different geographic areas (Forshaw, 2006). For all of these species, the use of multiple habitat types likely precludes the opportunity for habitat structure to exert consistent selective pressure on the evolution of their calls.

More generally, this study does illustrate the usefulness of remote sensing imagery in the study of ecological factors driving signal evolution. In most cases, the collection of habitat data is not feasible across multiple taxa or availability of pre-existing habitat descriptions is

limited and does not offer an objective measure of habitat structure.

Character displacement and signal evolution

We did not find an effect of species interactions on acoustic call parameters. Pairs of closely related species found in sympatry did not show greater difference in their calls than those in allopatry. Tobias *et al.* (2014) recently showed in ovenbirds that the absence of the predicted pattern by character displacement across large radiations is due to the influence of evolutionary time through processes of genetic drift or adaptation rather than species interactions. It would be interesting to test the influence of lineage age on the evolution of Neotropical parrot contact calls, but doing so would require a larger sample of lineages than found in the present study.

Conclusions

This study shows strong support for the influence of morphological traits shaping acoustic signals in parrots. We found common general patterns of association between morphology and call structure found in previous studies. However, some generalizations on the influence of morphology on song evolution derived from other avian taxa, such as an effect of bill length on frequency range, were not supported in parrots. Our results further suggest that habitat structure has not been a uniformly important selective pressure in the evolution of parrot contact calls, although further investigation is warranted. More importantly, our results suggest that life-long learned acoustic signals can evolve at a similar pace to morphological traits. These results show that cultural evolution does not necessarily accelerate the pace of evolutionary change of behavioural traits such as acoustic signals.

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Author contribution

AMG and MAS contributed equally to this paper; all authors contributed to the data collection, analysis, writing and reviewing of the manuscript.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Ultrametric tree of the 51 species included in the study from the tribe Arini from Schirtzinger

(2011) generated by a correlated model of substitution (Paradis, 2013).

Table S1 Source, catalogue number, date, recordist, and country of contact call recordings used in the study.

Table S2 Mean measurements of all acoustic parameters, vegetation index (EVI), body mass, and bill length for each species.

Table S3 Regression models of acoustic parameters with body mass, bill length, and vegetation index ($n = 51$ taxa) controlling for phylogenetic effects.

Table S4 Results of pairwise comparisons of evolutionary rates for acoustic parameters, body mass, bill length, and vegetation index for 51 species of the tribe Arini.

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