RESEARCH ARTICLE

Dissecting the roles of body size and beak morphology in song evolution in the "blue" cardinalids (Passeriformes: Cardinalidae)

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ABSTRACT
Certain vocal signals can be difficult or challenging to produce due to constraints on vocal mechanics. In the case of birdsong, both body size and beak morphology have been shown to constrain spectral and temporal song parameters. Therefore, variation within a clade in both these morphological traits could exert an important effect in vocal evolution and diversity. However, given the allometric relationship between body size and beak size, it is not always easy to separate the effects they may have. Additionally, different habitats impose different sources of sound attenuation and degradation, and birdsong seems to be adapted to minimize habitat-specific sound degradation. Here, we analyze vocal variation in relation to both body size and beak morphology in 13 of 14 species in the "blue" clade of the Cardinalidae, controlling for habitat characteristics. On one hand, we found the predicted negative correlation of body size with song frequencies, but no effect of body size on temporal variables. On the other hand, we found no relationship between different beak dimensions (corrected by body size) and song frequencies, but we found that beak length was correlated negatively with note rate and positively with the duration of the interval between notes. Similar results have been found previously, but mostly in species with trilled songs, in which notes are equal and produced at a constant rate. Our results show that different morphological variables, even if strongly correlated, can exert differential constraints in a complex behavioral trait such as song. A better understanding of the relevance of these proximate factors in shaping vocal evolution is fundamental to understanding the causes of avian vocal diversity.

Keywords: beak morphology, birdsong, body size, constraints, evolution, habitat

Separando los roles del tamaño corporal y la morfología del pico en la evolución del canto en los cardinalinos "azules" (Passeriformes: Cardinalidae)

RESUMEN
Ciertas señales vocales pueden ser difíciles de producir debido a restricciones en los mecanismos vocales. En el caso del canto de las aves, se ha visto que tanto el tamaño corporal como la morfología del pico restringen parámetros espectrales y temporales del canto. Por lo tanto, la variación dentro de un clado en ambos caracteres morfológicos puede ejercer un importante efecto en la evolución vocal y su diversidad. Sin embargo, dada la relación alométrica entre el tamaño del cuerpo y del pico, no siempre es fácil separar los efectos que cada uno pueda tener. Además, diferentes hábitats imponen diferentes fuentes de atenuación y degradación del sonido, y el canto de las aves parece haberse adaptado a minimizar la degradación acústica específica a su hábitat. Aquí analizamos la variación vocal en relación tanto al tamaño corporal como a la morfología del pico en 13 de las 14 especies del clado "azul" de la familia Cardinalidae, controlando por las características del hábitat. Por un lado, encontramos la correlación negativa predicha entre el tamaño corporal y las frecuencias del canto, pero ningún efecto del tamaño corporal en las variables temporales. Por otro lado, no encontramos ninguna relación entre las dimensiones del pico (corregidas por el tamaño corporal) y las frecuencias del canto, pero encontramos que el largo del pico correlaciona negativamente con la tasa de notas y positivamente con la duración del intervalo entre notas. Previamente se encontraron resultados similares, pero principalmente en especies con trinos, donde las notas son iguales y producidas a tasa constante. Nuestros resultados muestran que diferentes variables morfológicas, incluso si están fuertemente correlacionadas, pueden ejercer diferentes restricciones en un carácter comportamental complejo como es el canto. Un mejor entendimiento de la relevancia de estos factores próximos en la evolución vocal es fundamental para entender las causas de la diversidad vocal aviar.

Palabras clave: canto de las aves, hábitat, morfología del pico, restricciones, tamaño corporal

INTRODUCTION

Despite fulfilling virtually the same functions in all species studied so far, song exhibits an extraordinary level of diversification among birds (Catchpole and Slater 2008). The study of song evolution has traditionally been focused on sexual selection and cultural transmission as factors that promote vocal diversity (Podos et al. 2004a), with a particular focus on species from temperate habitats (Podos 2013). However, studying the mechanistic constraints on song production is also key to understanding the causes of vocal diversity, because those constraints can limit the direction or degree of evolutionary change that is promoted by selective factors or random processes.

Birdsong can be considered as a set of multiple characters (Gil and Gahr 2002) that can be subject to constraints imposed by different morphological traits. The fundamental frequencies of vocalizations are determined by the oscillation frequency of certain membranes in the syrinx (Goller and Larsen 1997a, 1997b, Larsen and Goller 1999). Because the size of the structures of the syrinx covary with body size (Riede and Goller 2014), theory predicts that birds of larger body size will produce sounds with longer wavelengths (i.e. of lower frequency; Bradbury and Vehrencamp 1998, Riede and Goller 2014). A negative correlation between song frequencies and body size was found in multispecies comparisons of passeriforms (Wall-schläger 1980, Ryan and Brenowitz 1985, Seddon 2005, Price et al. 2006), pigeons (Tubaro and Mahler 1998), and tinamiforms (Bertelli and Tubaro 2002). Similar results were obtained when comparing closely related species or at the intraspecific level, both in passerines and non-passerines (Galeotti et al. 1997, Mager et al. 2007, Turčocová et al. 2010, Hall et al. 2013; but see Cardoso et al. 2008).

The resonance properties of the supra-siringeal structures also play an important role in sound production, with shorter vocal tracts emphasizing higher frequencies (Nowicki 1987, Hoese et al. 2000). Consistently, birds sing with their beaks more open when emphasizing higher frequencies (Hausberger et al. 1991, Westneat et al. 1993), and beak size is negatively correlated with song frequencies (Palacios and Tubaro 2000, Huber and Podos 2006) and song bandwidth (Huber and Podos 2006, Derryberry et al. 2012). Beak morphology also affects the temporal parameters of song, particularly the pace at which song notes are produced. For example, species or individuals with larger beaks produce trills (rapid succession of similar or equal notes in all or part of the song) with lower note rates (Podos 2001, Podos et al. 2004b, Huber and Podos 2006). Larger beaks are associated with a more robust jaw musculature, which allows greater bite force (van der Meij and Bout

2004) but, in turn, limits the speed at which the beak can be opened or closed to emphasize the different fundamental frequencies of the song (Herrel et al. 2009). Body size has been shown to be correlated with temporal aspects of song as well (García et al. 2014, Mason and Burns 2015). However, it remains unclear how body size might affect the temporal parameters of song.

Both song frequencies and temporal structure can also be strongly influenced by the habitats in which the birds communicate, which impose different sources of sound attenuation and degradation depending on their particular characteristics. Species of closed habitats (rainforests, woods) produce signals of lower frequency, with more separated notes than those of open environments like grasslands or savannas (Morton 1975, Ryan and Brenowitz 1985, Wiley 1991, Tubaro and Lijtmaer 2006). These differences are usually interpreted as adaptations to minimize the degradation of the signal traveling through its native environment (acoustic adaptation hypothesis; Morton 1975, Boncoraglio and Saino 2007). Therefore, it is fundamental to take habitat type into account when analyzing the effects of other factors in song evolution.

Here, we analyze vocal variation in relation to different morphological traits in a group of closely related passerines, the "blue" clade of Family Cardinalidae (sensu Klicka et al. 2007; hereafter "blue cardinalids"). The blue cardinalids are a widespread group of New World songbirds of the genera *Amaurospiza*, *Cyanocompsa*, *Cyanoloxia*, and *Passerina*, which, like most cardinalids, are well known for producing rich and complex vocalizations (Ridgely and Tudor 1989, Orenstein and Brewer 2011; see Appendix Figure 4). García et al. (2014) found that body-size differences were related to song variation among 3 of the species in the clade. Our objective was to test whether body size and beak morphology have had an effect in shaping birdsong within this group, and to determine which of the different aspects of vocal phenotype were affected by them.

METHODS

Acoustic Analyses

We used recordings in "wav" or "aiff" format, digitized at a rate of 44.1 kHz, obtained from sound collections or commercial compilations or provided personally by the recordists (see Supplemental Material Table S1). We analyzed one song per individual from 13 of the 14 species in the clade (except the relatively recently described *Amaurospiza carrizalenzis*; Lentino and Restall 2003), covering most of the species ranges and their subspecies (mean $\pm$ SD of individuals per species: 21.85 $\pm$ 21.32; range: 2–73).

Recordings were analyzed in Raven 1.4 (Bioacoustics Research Program 2011). Spectrograms were generated using the following parameters: window type Hann, window size and Fourier transform of 512 samples, 50% overlap (resulting in a hop size of 256 samples), and grid spacing 86.1 Hz. On each spectrogram, we selected a song (if there was more than one) on the basis of sound quality, considering signal-to-noise ratio, echoes, and/or overlapping signals. We manually delimited the beginning and end of the song and its maximum and minimum frequencies, as well as the beginning and end of the notes (considering a "note" any continuous vocalization on the spectrogram, or discontinuous vocalizations separated by an interval ≤0.015 s). We used cursor measurements instead of automated algorithms or methods based on a standard decibel threshold (Zollinger et al. 2012) because of the heterogeneity of the recording dataset and the high variation in the sound quality of the recordings. We then measured 4 spectral variables: (1) minimum frequency (MinF, the lowest frequency of the entire song), (2) maximum frequency (MaxF, the highest frequency of the entire song), and (3) emphasized frequency (EmpF, the frequency with greatest amplitude in the song, obtained using the "Max Frequency" option in Raven 1.4). We calculated (4) bandwidth (Band) as the difference between MaxF and MinF. We also calculated 5 temporal parameters: (5) song duration (SongD, the time elapsed from the beginning of the first note to the end of the last note), (6) number of notes (NNotes), (7) mean note duration (NoteD, the time elapsed from the beginning to the end of a note, averaged over all notes in a song), (8) mean duration of interval between notes (InterNoteD, the time elapsed between the end of a note and the beginning of the next one, averaged over all the intervals of a song), and (9) note rate (NoteR, the number of notes per second, calculated as NNotes/SongD).

Morphology

We used body mass as a proxy of body size for our analyses. Although these 2 parameters are not necessarily directly correlated, body mass is the preferred univariate measure of body size in birds (Freeman and Jackson 1990). To calculate mean body mass of each species (Figure 1), body-mass data of males of each species (mean ± SD of individuals per species: 68.15 ± 76.76; range: 3–267) were requested from various institutions (see Supplemental Material Table S2) or obtained from the literature (Belton 1985, Reinert et al. 1996, Verea et al. 1999) and through the VertNet portal (http://www.vertnet.org; see also Supplemental Material Table S2).

We obtained beak measurements from study skins of 4 ornithological collections (see Supplemental Material Table S3), also covering most of the species ranges and
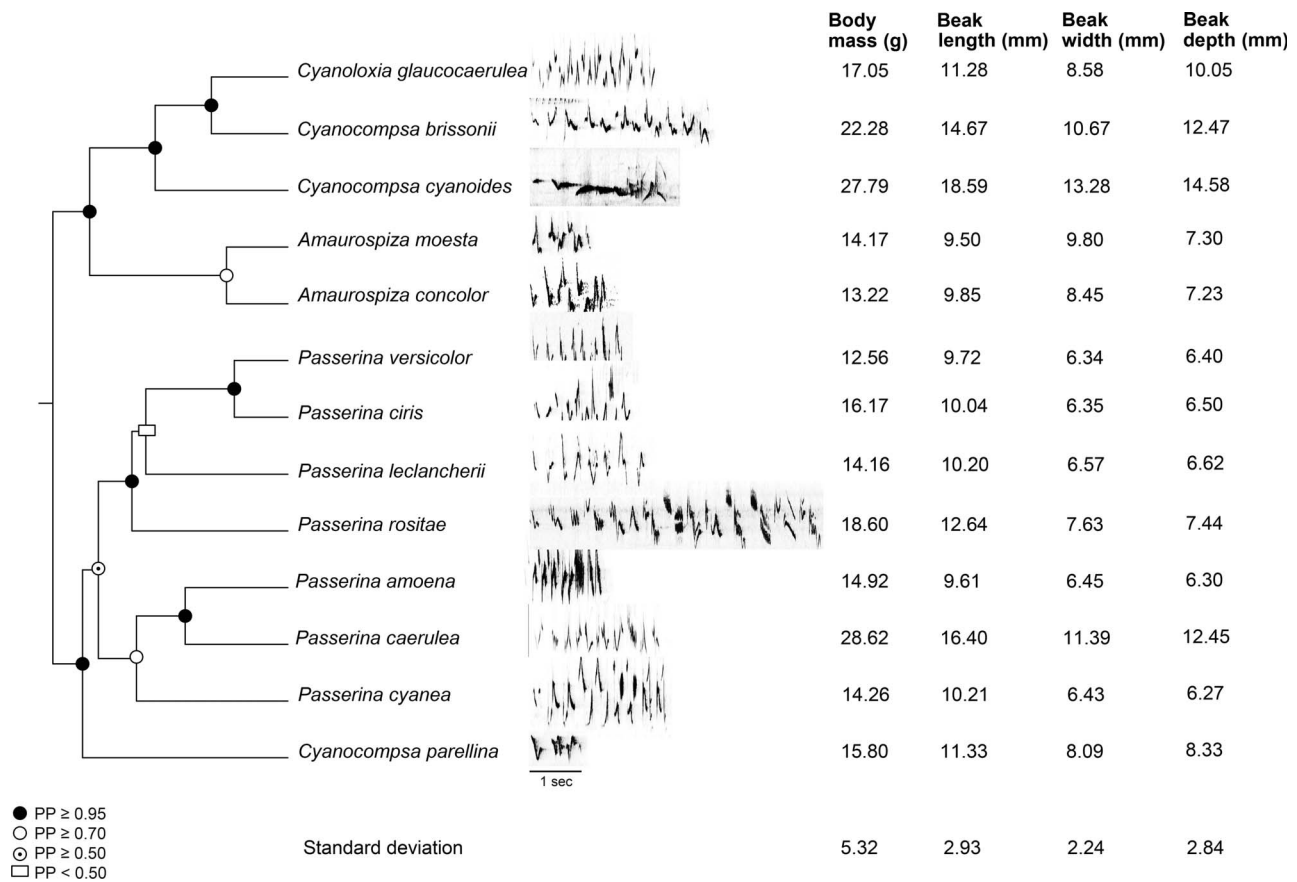
their subspecies (mean ± SD of individuals per species: 14.56 ± 9.96; range: 6–43). We selected adult male specimens and used a digital caliper (ROK model DC-122A, resolution 0.01 mm) to measure (1) beak length, (2) beak width, and (3) beak depth (following Baldwin et al. 1931).

Because body mass and beak measurements were obtained from different specimens, we used the natural logarithm of the mean values per species (Figure 1) to first evaluate whether they were correlated. We used phylogenetic generalized least squares regressions (PGLS; see Martins and Hansen 1997) based on the species tree reconstructed by Bryson et al. (2014) from a multilocus dataset (see Figure 1). We used the "gls" function of the "nlme" package (Pinheiro et al. 2016) implemented in R 3.3.2 (R Core Team 2016). Because we obtained significant results in the 3 cases ($\beta > 0.70$, $P < 0.001$ in all cases), we then calculated the phylogenetic residuals (Revell 2009) of beak length, width, and depth on body mass using the "phytools" package (Revell 2012) implemented in R.

Comparative Analyses

We used PGLS regressions to evaluate the correlation of each of the 9 acoustic parameters with body mass and the phylogenetic residuals of each of the 3 beak measurements. The acoustic variables were also natural log-transformed prior to the analyses. We used the tree topology and branch lengths of the species tree reconstructed by Bryson et al. (2014) from a multilocus dataset (see Figure 1) and calculated the PGLS models using the "gls" function of the "nlme" package (Pinheiro et al. 2016) for R. We considered a Brownian-motion model of character evolution and fitted the model by the maximum likelihood method. We are aware that considering only a Brownian-motion model of evolution is a limitation of our study, but we decided to do so because of the small number of compared species. It has been shown that the best approach is adjusting a PGLS model while simultaneously estimating Pagel's lambda, but this was proved using simulations with large sample sizes (100 species; Revell 2010). The significance of lambda is very sensitive to the number of species and may perform poorly as a measure of phylogenetic signal at small sample sizes (Münkemüller et al. 2012). Therefore, we preferred to only test and report the results obtained based on the simplest model of Brownian motion.

To control for a possible effect of habitat characteristics, we assigned each species to one of 3 habitat types (open, semi-open, or closed) according to the primary environment designated for each of them according to Stotz et al. (1996). In total, 3 species were assigned to an open habitat (*Passerina amoena*, *P. ciris*, and *P. versicolor*),

	Body mass (g)	Beak length (mm)	Beak width (mm)	Beak depth (mm)
Cyanoloxia glaucocaerulea	17.05	11.28	8.58	10.05
Cyanocompsa brissonii	22.28	14.67	10.67	12.47
Cyanocompsa cyanoides	27.79	18.59	13.28	14.58
Amaurospiza moesta	14.17	9.50	9.80	7.30
Amaurospiza concolor	13.22	9.85	8.45	7.23
Passerina versicolor	12.56	9.72	6.34	6.40
Passerina ciris	16.17	10.04	6.35	6.50
Passerina leclancherii	14.16	10.20	6.57	6.62
Passerina rositae	18.60	12.64	7.63	7.44
Passerina amoena	14.92	9.61	6.45	6.30
Passerina caerulea	28.62	16.40	11.39	12.45
Passerina cyanea	14.26	10.21	6.43	6.27
Cyanocompsa parellina	15.80	11.33	8.09	8.33
Standard deviation	5.32	2.93	2.24	2.84

● PP ≥ 0.95
○ PP ≥ 0.70
◉ PP ≥ 0.50
▢ PP < 0.50

1 sec

FIGURE 1. Tree topology used for phylogenetic generalized least squares regressions, based on the species tree reconstructed by Bryson et al. (2014) using a multilocus dataset. Mean values per species and standard deviation of the morphological predictors are shown, along with a representative song for each species. Spectrograms are based on recordings from the Cornell University Macaulay Library (CUML): *Amaurospiza concolor* (CUML 130404); *A. moesta* (CUML 20247); *Cyanocompsa brissonii* (CUML 171724); *C. cyanoides* (CUML 55262); *Cyanoloxia glaucocaerulea* (CUML 67599); *Cyanocompsa parellina* (CUML 184333); *Passerina amoena* (CUML 106549); *P. caerulea* (CUML 105519); *P. ciris* (CUML 105233); *P. cyanea* (CUML 26418); *P. leclancherii* (CUML 16952); *P. rositae* (CUML 55473); and *P. versicolor* (CUML 16954). Note: Bryson et al. (2014) found that *A. concolor* was paraphyletic and that *A. c. aequatorialis* was more closely related to *A. moesta*. Because all the analyzed recordings of *A. concolor* but one are *A. c. aequatorialis*, we used the corresponding branch length.

5 species to a semi-open habitat (*Cyanocompsa brissonii*, *Cyanoloxia glaucocaerulea*, *P. caerulea*, *P. cyanea*, and *P. leclancherii*), and 5 species of to a closed habitat (*Amaurospiza concolor*, *A. moesta*, *Cyanocompsa cyanoides*, *C. parellina*, and *P. rositae*). Then each of the 36 base linear models (acoustic variable ∼ morphological trait) was compared to the other 2 models that included habitat type as an additive or interactive factor. For each, we identified the best-fitting model on the basis of Akaike's Information Criterion (AIC) corrected for small sample size (AIC$_c$), which we calculated using the "MuMIn" package (Barton 2016) for R. Following Burnham et al. (2011), we also calculated the differences of each compared model with the one with lowest AIC$_c$ value (ΔAIC$_c$) and considered the ones with ΔAIC$_c$ < 6 as competing models. We visually inspected the distribution of residuals of the best-fitting model and

performed a Lilliefors test on the standardized residuals to evaluate whether they were normally distributed.

RESULTS

In all cases, the best-fitting model was the one not including habitat type as an additive or interactive factor (Table 1). In some cases, the model including habitat type as an additive factor could be considered competitive, but in all of these cases the effect of habitat type was not significant ($P ≥ 0.06$), with the exception of MinF ∼ body mass × habitat type (see Supplemental Material Table S4). Body mass showed a significant negative correlation with both MinF and MaxF, and also with EmpF (Table 2 and Figure 2A–2C). None of the temporal variables here considered showed a relationship with body mass, and neither of the spectral variables showed a relationship with any of the beak measurements

TABLE 1. Comparison of model fit (relationship of acoustic variables with each morphological trait alone, or with habitat type as an additive or interactive factor) according to ΔAIC_c values.

	Model		
Acoustic variable [a]	Body mass	Body mass + habitat	Body mass × habitat
MinF	0	8.51	1.97
MaxF	0	6.35	24.00
Band	0	6.05	23.14
EmpF	0	8.28	17.82
SongD	0	9.02	22.53
NoteD	0	7.13	22.15
InterNoteD	0	4.48	18.96
NNotes	0	8.23	25.73
NoteR	0	6.34	19.70
	Model		
Acoustic variable [a]	Beak depth	Beak depth + habitat	Beak depth × habitat
MinF	0.00	8.49	23.37
MaxF	0.00	5.21	21.59
Band	0.00	5.04	20.48
EmpF	0.00	7.04	24.63
SongD	0.00	8.36	20.89
NoteD	0.00	5.80	20.65
InterNoteD	0.00	5.31	20.16
NNotes	0.00	8.45	22.30
NoteR	0.00	5.13	18.55
	Model		
Acoustic variable [a]	Beak width	Beak width + habitat	Beak width × habitat
MinF	0.00	7.74	21.99
MaxF	0.00	6.27	21.37
Band	0.00	6.15	20.00
EmpF	0.00	7.71	23.86
SongD	0.00	7.48	16.28
NoteD	0.00	6.51	23.28
InterNoteD	0.00	5.41	19.85
NNotes	0.00	9.20	22.33
NoteR	0.00	5.82	20.91
	Model		
Acoustic variable [a]	Beak length	Beak length + habitat	Beak length × habitat
MinF	0.00	7.52	22.81
MaxF	0.00	4.91	21.50
Band	0.00	4.32	20.03
EmpF	0.00	7.56	24.86
SongD	0.00	8.57	24.81
NoteD	0.00	7.64	18.48
InterNoteD	0.00	6.04	20.50
NNotes	0.00	8.56	18.30
NoteR	0.00	7.06	17.23

[a] MinF = minimum frequency; MaxF = maximum frequency; Band = bandwidth; EmpF = emphasized frequency; SongD = song duration; NoteD = mean note duration; InterNoteD = mean duration of interval between notes; NNotes = number of notes; NoteR = note rate.

(see Appendix Figures 5–8). However, InterNoteD showed a significant positive correlation with beak length (Table 2 and Figure 3A), whereas NoteR showed a significant negative correlation with this beak dimension (Table 2 and Figure 3B). The results for MinF should be interpreted carefully, because the model residuals were not normally distributed according to the Lilliefors test, and alternative transformations did not correct this.

TABLE 2. Results from phylogenetic generalized least squares regressions of the best-fitting models. Effect size and its standard error (β ± SE) and statistical significance are shown. Significant results are in bold.

Acoustic variable [a]	Body mass β ± SE	Body mass P	Beak depth β ± SE	Beak depth P	Beak width β ± SE	Beak width P	Beak length β ± SE	Beak length P
MinF	**−0.23 ± 0.10**	**0.039**	−0.42 ± 0.28	0.161	−0.25 ± 0.29	0.394	−0.67 ± 0.39	0.113
MaxF	**−0.42 ± 0.16**	**0.021**	−0.27 ± 0.50	0.598	−0.53 ± 0.46	0.276	−0.27 ± 0.72	0.717
Band	−0.49 ± 0.23	0.061	−0.25 ± 0.69	0.723	−0.69 ± 0.63	0.301	−013 ± 0.99	0.899
EmpF	**−0.42 ± 0.10**	**0.001**	−0.56 ± 0.36	0.150	−0.47 ± 0.36	0.213	−0.74 ± 0.53	0.188
SongD	0.34 ± 0.32	0.310	0.02 ± 0.84	0.985	−0.74 ± 0.78	0.362	1.32 ± 1.14	0.269
NoteD	0.32 ± 0.25	0.214	−0.26 ± 0.66	0.698	0.33 ± 0.63	0.603	1.44 ± 0.84	0.113
InterNoteD	0.10 ± 0.31	0.748	0.61 ± 0.76	0.439	0.57 ± 0.73	0.455	**2.33 ± 0.87**	**0.021**
NNotes	0.11 ± 0.34	0.757	0.01 ± 0.87	0.987	−1.12 ± 0.76	0.171	−0.37 ± 1.23	0.770
NoteR	−0.24 ± 0.24	0.328	−0.03 ± 0.63	0.963	−0.39 ± 0.59	0.519	**−1.73 ± 0.73**	**0.037**

[a] MinF = minimum frequency; MaxF = maximum frequency; Band = bandwidth; EmpF = emphasized frequency; SongD = song duration; NoteD = mean note duration; InterNoteD = mean duration of interval between notes; NNotes = number of notes; NoteR = note rate.

DISCUSSION

We found evidence that bird morphology had a role in shaping song variation among the blue cardinalids. In particular, variation in spectral parameters is correlated with body size, while the pace of note production is related to beak length. Notably, our results also showed that song evolution within this group has been independent of habitat type.

Using body mass as a proxy body-size estimator, we found that song maximum, minimum, and emphasized frequencies decreased as body size increased. This is congruent with previous results in both passerines and non-passerines (Ryan and Brenowitz 1985, Galeotti et al. 1997, Tubaro and Mahler 1998, Bertelli and Tubaro 2002, Seddon 2005, Price et al. 2006, Mager et al. 2007, Hall et al. 2013, Mason and Burns 2015) and demonstrates that an influence of body size on song can be detected even between species with moderate size variation. It is particularly relevant to find such results among oscine passerines, given that it has been argued elsewhere that song learning could "mask" the constraint imposed by morphology (see, e.g., Cardoso et al. 2008). However, if the syrinx is constrained to produce certain types of sounds (e.g., with frequencies limited to a given range by the size of the vibrating membranes), the space of potential song phenotypes will be limited whether the instructions sent by the nervous system are innate or learned.

We found no relationship between song frequencies and any of the beak measurements we considered. Beak-size variation in this group is not as extreme as in other groups previously studied (e.g., dendrocolaptids; see Palacios and Tubaro 2000). It is possible that small beak-size differences have no influence on spectral aspects of song, contrary to what happens with temporal parameters (see below).

Variation in temporal aspects of song has traditionally been related to the effect of habitat on its evolution (Morton 1975, Ryan and Brenowitz 1985, Wiley 1991, Tubaro and Lijtmaer 2006). However, habitat type was not related to any of the acoustic parameters analyzed here, in accordance with recent findings in other avian families (see, e.g., Mason and Burns 2015). By contrast, we found that note rate is related to the length of the beak. Beak size has been shown to limit the rate of note production in trilled songs (i.e. with series of notes or groups of notes repeated at a constant rate), both in oscines (Podos 2001) and in suboscines (Derryberry et al. 2012). The results presented here are the first, to our knowledge, to show a relationship similar to that initially found by Podos (2001) between beak size and temporal structure of song (specifically on rate of note production) but in species with vocalizations in which the notes are not equal and do not occur at regular intervals as in a trill (but for an example at the intraspecific level in partially trilled songs, see Badyaev et al. 2008). This is relevant to the study of song's relationship to mate choice and sexual selection in a wider range of bird species, because producing songs with higher note rates can be considered more demanding in terms of vocal performance and, therefore, a way to communicate individual quality (Ballentine et al. 2004).
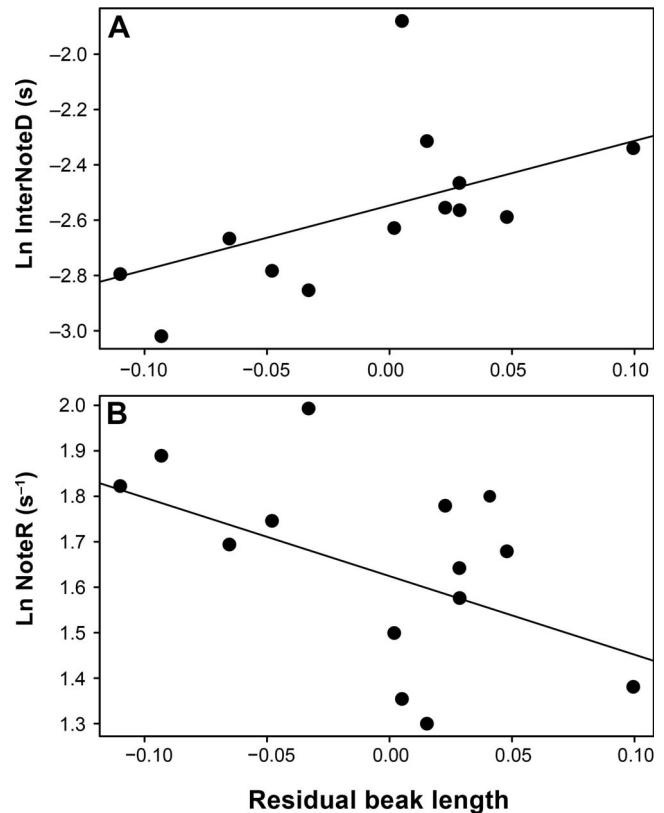
Of the 3 beak dimensions considered in our analyses, only beak length showed a significant correlation with song temporal traits. The functional explanation for a negative correlation between beak size and note rate would be the trade-off between bite force and beak movement speed. When comparing 9 species of Darwin's finches, Herrel et al. (2005) found that bite force was correlated strongly with beak depth and width but only weakly with beak length. Thus, we would have expected a stronger correlation of note rate with beak width and/or depth rather than length. A possible explanation is that only beak
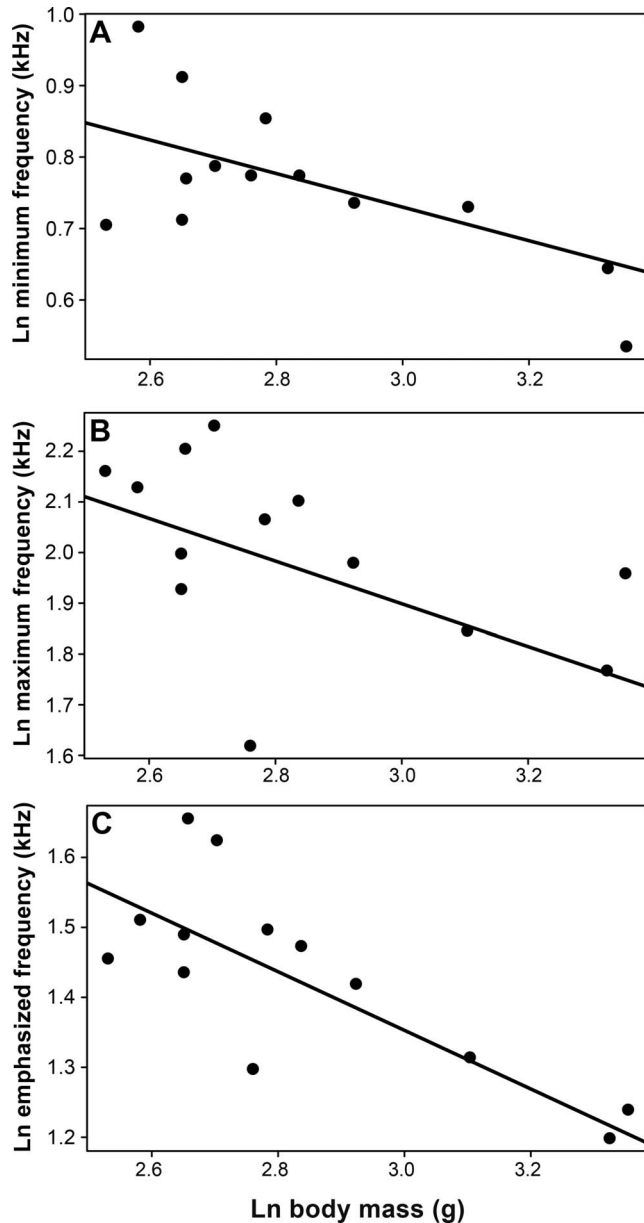
FIGURE 2. Scatterplot of the 3 spectral variables that were correlated with body mass.

FIGURE 3. Scatterplot of the 2 temporal variables that were correlated with beak length.

length showed a significant correlation with song traits not for a functional reason, but because it is the dimension that showed the largest variation among species (see Figure 1) in a group with relatively small variation in beak size. However, given that the difference in variability is small, this should be interpreted carefully.

Previous studies at different taxonomic scales found that body mass was correlated with certain temporal parameters of song, such as the duration of the notes, the pauses between notes, or the note rate (García et al. 2014, Mason and Burns 2015). However, it remained unclear what functional relationship could explain such correlations

between body size and the temporal structure of song. Although we could directly differentiate the effects of body and beak morphology, beak size normally shows a strong correlation with body size. Therefore, such results may actually be due to differences in beak morphology (not considered in those studies) being reflected by the differences in body size.

In summary, our results show that different morphological traits affect the evolution of different song parameters and that beak morphology constrains the rate of note production, even when the notes are not part of a trill as in the species in which this relationship was previously studied (Podos 2001). These morphological constraints, which can arise as a result of directional or random evolutionary processes on nonvocal traits, can explain vocal variation among closely related species, and their study is promising in terms of understanding how birdsong can convey information on individual quality.

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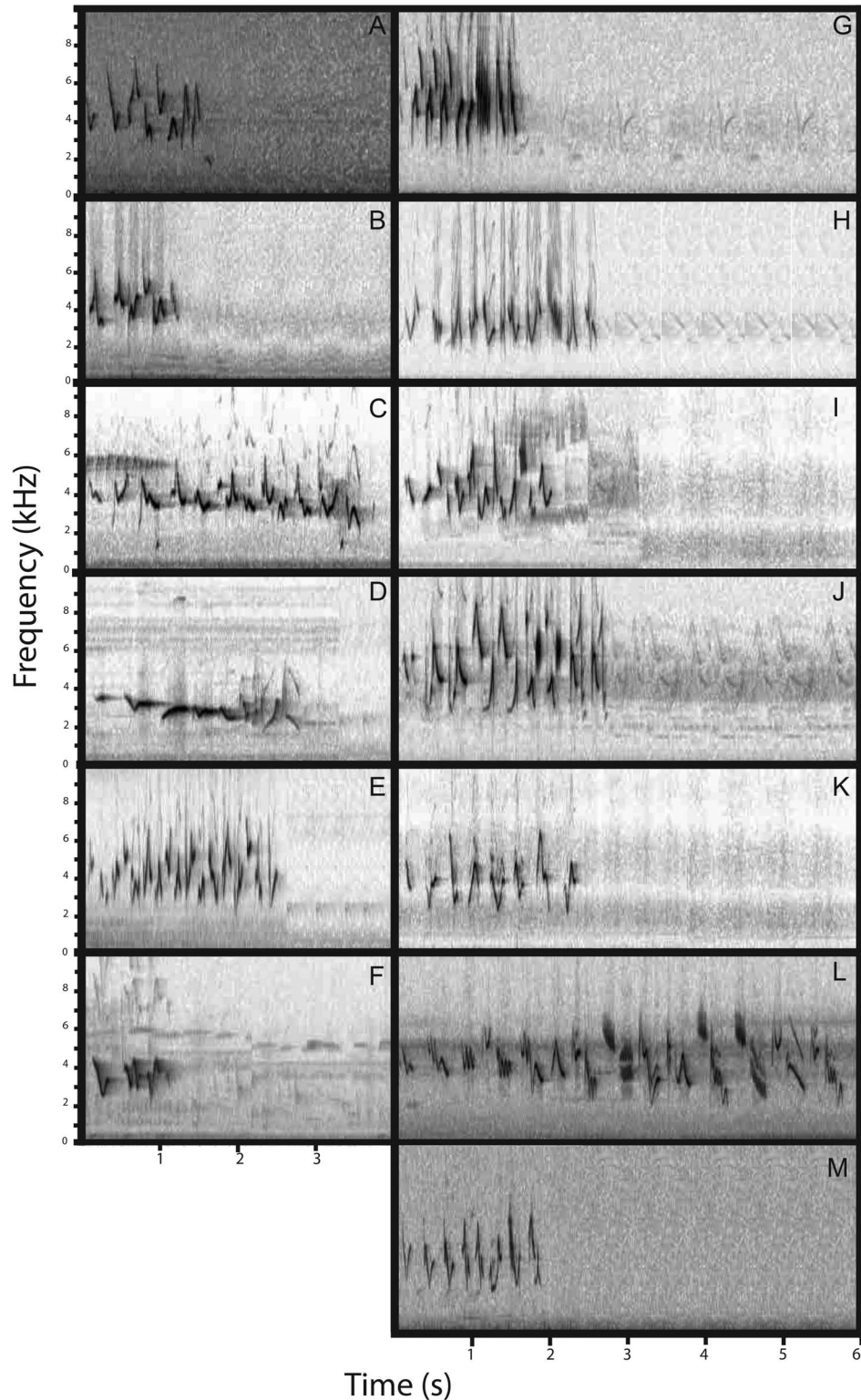
APPENDIX FIGURE 4. Examples of songs of the blue cardinalids, based on recordings from the Cornell University Macaulay Library (CUML): (**A**) *Amaurospiza concolor* (CUML 130404); (**B**) *A. moesta* (CUML 20247); (**C**) *Cyanocompsa brissonii* (CUML 171724); (**D**) *C. cyanoides* (CUML 55262); (**E**) *Cyanoloxia glaucocaerulea* (CUML 67599); (**F**) *Cyanocompsa parellina* (CUML 184333); (**G**) *Passerina amoena* (CUML 106549); (**H**) *P. caerulea* (CUML 105519); (**I**) *P. ciris* (CUML 105233); (**J**) *P. cyanea* (CUML 26418); (**K**) *P. leclancherii* (CUML 16952); (**L**) *P. rositae* (CUML 55473); and (**M**) *P. versicolor* (CUML 16954).
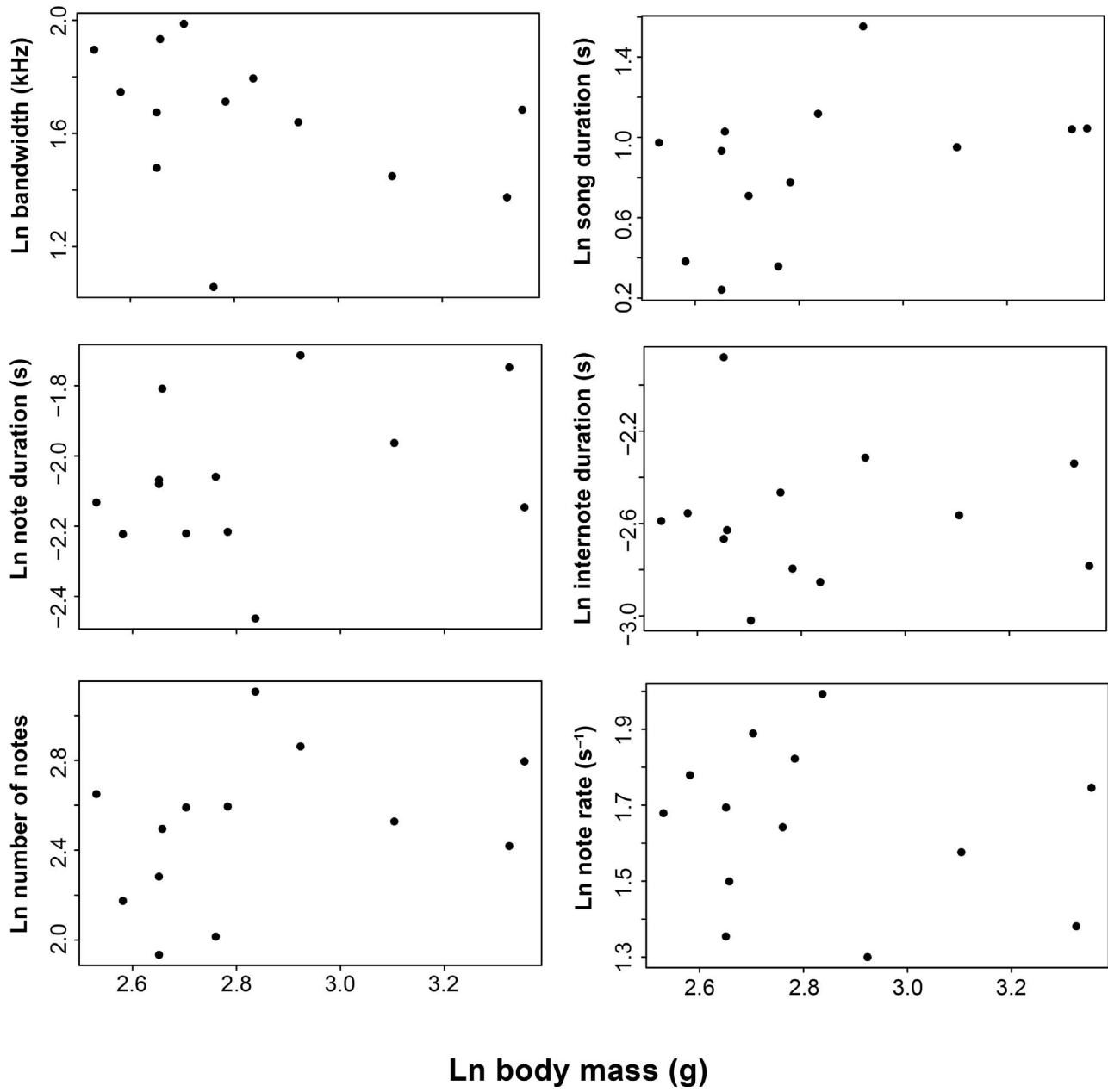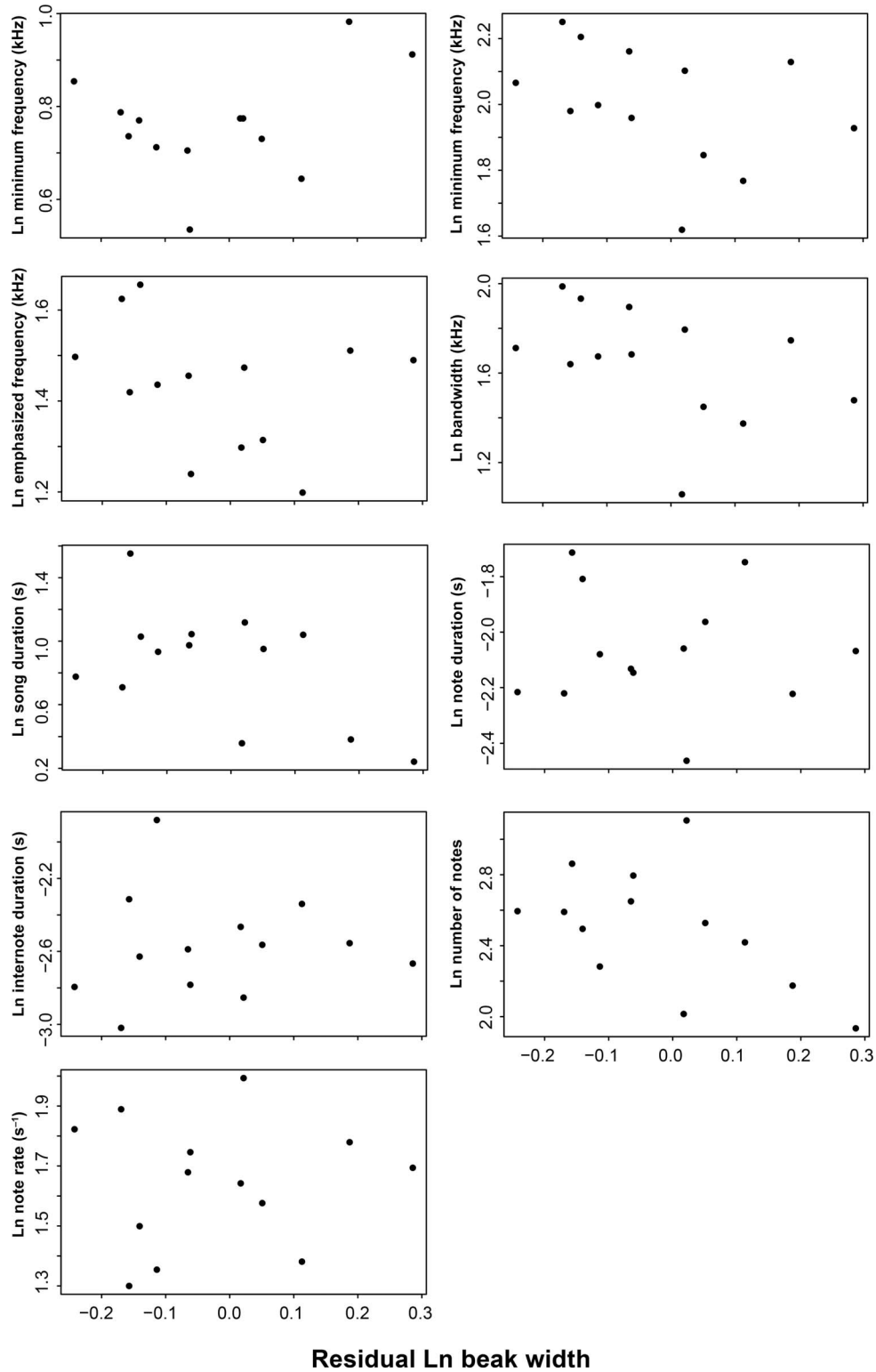
APPENDIX FIGURE 5. Scatterplot of the acoustic variables that showed no significant correlation with body mass.
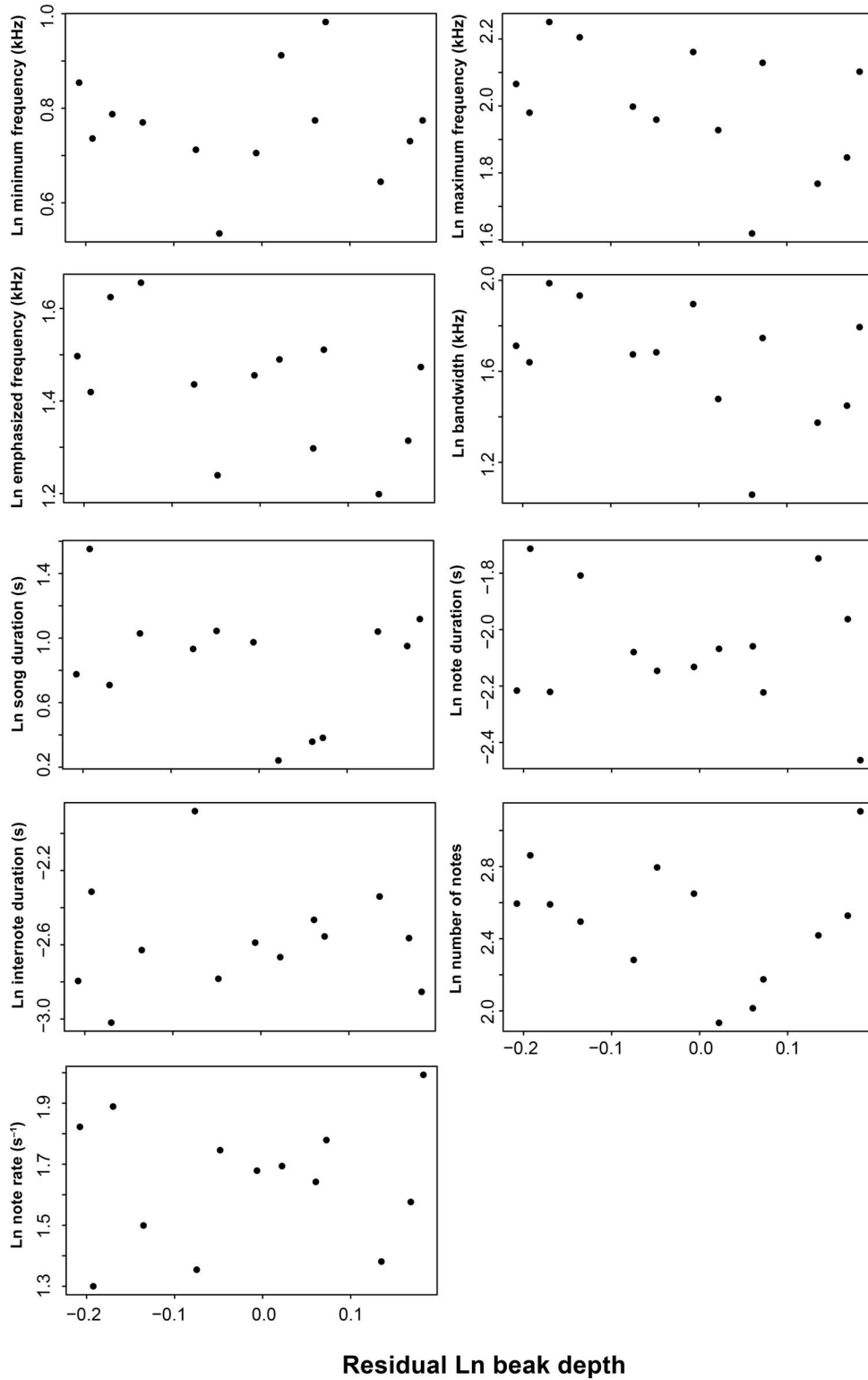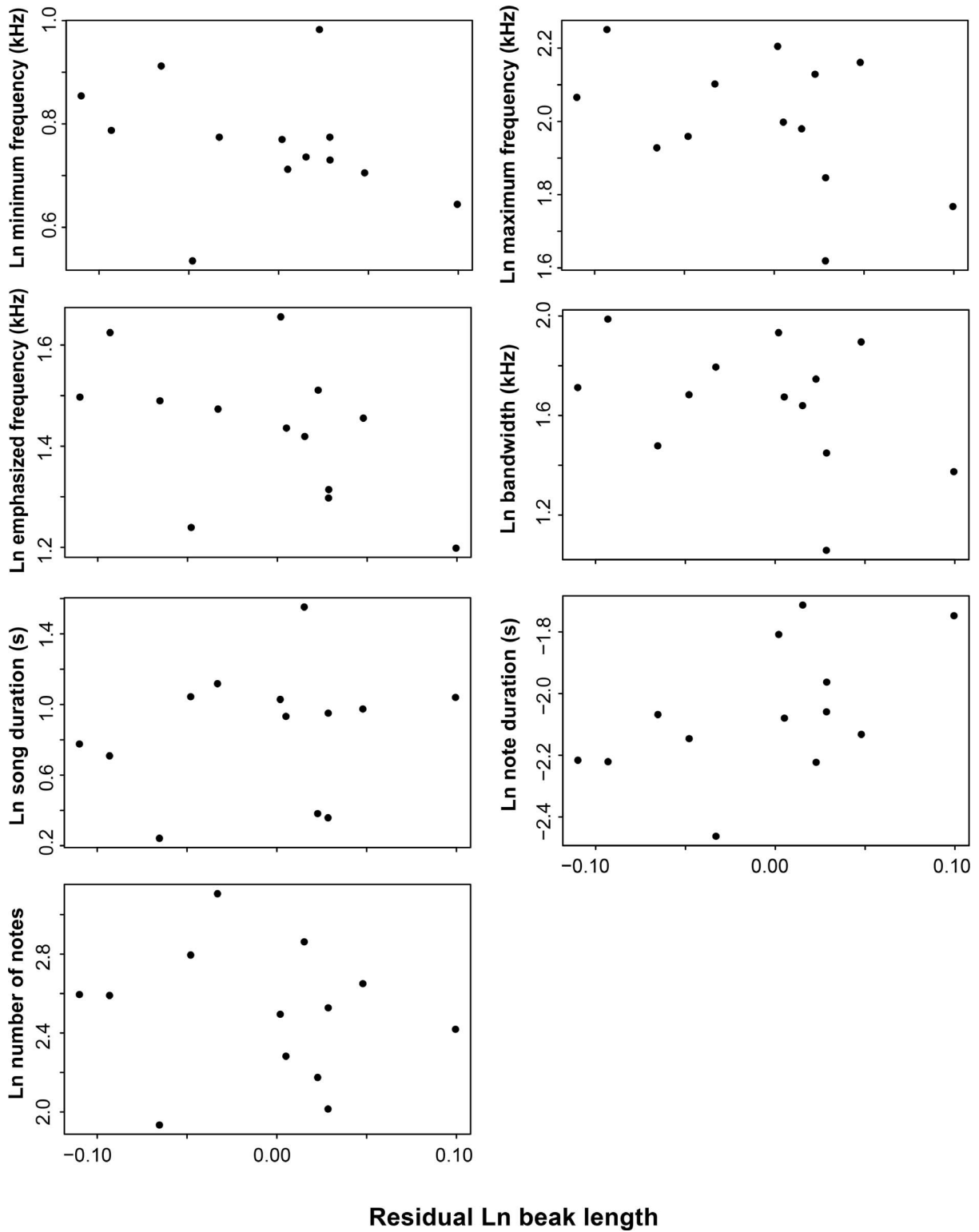
APPENDIX FIGURE 6. Scatterplot of the acoustic variables that showed no significant correlation with beak depth.

APPENDIX FIGURE 7. Scatterplot of the acoustic variables that showed no significant correlation with beak width.

APPENDIX FIGURE 8. Scatterplot of the acoustic variables that showed no significant correlation with beak length.