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Intraspecific and interspecific vocal variation in three Neotropical cardinalids (Passeriformes : Fringillidae) and its relationship with body mass

Natalia C. García[A,C], *Ana S. Barreira*[A], *Cecilia Kopuchian*[A,B] *and Pablo L. Tubaro*[A]

[A]División de Ornitología, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Avenida Ángel Gallardo 470 (C1405DJR), Buenos Aires, Argentina.
[B]Centro de Ecología Aplicada del Litoral (CECOAL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ruta 5, km 2.5, Casilla de correo 291, 3400, Corrientes, Argentina.
[C]Corresponding author. Email: ngarcia@macn.gov.ar

Abstract. Several studies, of a large number of bird species, have shown that song frequencies have a strong negative relationship with body size. However, mixed results were obtained at the intraspecific level or when comparing closely related species. Here, we compared the vocalisations of the monotypic Glaucous-blue Grosbeak (*Cyanoloxia glaucocaerulea*), three of five subspecies of the Ultramarine Grosbeak (*Cyanocompsa brissonii*) and all four subspecies of the Blue-black Grosbeak (*Cyanocompsa cyanoides*). These taxa of Neotropical cardinalids differ significantly in body size, so we compared them to examine the allometric relationship of body size with song frequencies, using body mass as a proxy of body size. The maximum frequency and the emphasised frequency (i.e. the frequency of the song of highest amplitude) showed the expected negative correlation with body mass, supporting the idea that the aforementioned relationship stands when comparing closely related lineages in *Cyanoloxia* and *Cyanocompsa*. We also found that the duration of notes, rate of production of notes and duration of inter-note interval correlated with body mass: heavier species had longer notes produced at a lower rate and separated by longer intervals. The correlation of temporal variables with body mass suggests that body size could also influence the temporal structure of song. Our findings highlight once again the importance of considering the role of avian morphology when studying the evolution of song, even if differences in frequency and temporal variables of songs are small.

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Introduction

Even though avian song may be a very plastic character, particularly in birds with vocal learning, sound production can be constrained by different aspects of avian morphology. In comparing 90 species of passerines, Wallschläger (1980) found a negative relationship between body mass and song frequency. The fundamental frequencies of the song are given by the oscillation frequencies of two membranes in the syrinx: the lateral and medial labia (Goller and Larsen 1997*a*, 1997*b*; Larsen and Goller 1999). This oscillation frequency depends on the size, shape and tension of the labia (Mindlin and Laje 2005; Riede and Goller 2010), and the covariation of size of the syrinx structure with body size would explain the relationship of song frequencies with body size (Wallschläger 1980; Baptista 1996; Lambrechts 1996).

The same allometric relationship of song frequency with body size has been found in other species of passerines (Ryan and Brenowitz 1985; Badyaev and Leaf 1997; Seddon 2005; Price *et al*. 2006; Turčocová *et al*. 2010; Martin *et al*. 2011) and also non-passerines (Appleby and Redpath 1997; Tubaro and Mahler 1998; Bertelli and Tubaro 2002; Mager *et al*. 2007; Patel *et al*. 2010; Martin *et al*. 2011). Some of these studies examined the relationship of song frequencies with different morphological traits, such as total length of the bird or tarsal length rather than body mass, but all traits were used as proxies of body size to see if this aspect of avian morphology influenced vocal evolution.

Although the negative relationship between frequencies and body size seems to be well established in comparisons across a large number of species, mixed results were obtained in studies at the intraspecific level. Appleby and Redpath (1997) found that larger individuals of Tawny Owl (*Strix aluco*) produced lower frequencies, in accordance with the expected body size–song frequency allometry described above. The same was found in other species, such as the Common Loon (*Gavia immer*; Mager *et al*. 2007), Bluethroat (*Luscinia svecica*; Turčocová *et al*. 2010) and Black Swan (*Cygnus atratus*; Patel *et al*. 2010). However, larger subspecies of the Sharp-beaked Ground-Finch (*Geospiza difficilis*; Bowman 1979) and Summer Tanager (*Piranga rubra*; Shy 1983) produced songs of higher frequencies, and there was no relationship between song frequency and body size in male European Serins (*Serinus serinus*) and Dark-eyed Juncos (*Junco* *hyemalis*) (Cardoso *et al*. 2008). Cardoso *et al*. (2008) suggested that because differences in size among conspecifics are generally small the predicted differences in frequency are also small and thus possibly difficult to detect, if they occur.

The species of the tribe Cardinalini (Passeriformes : Fringillidae) are well known for producing rich and complex vocalisations (Ridgely and Tudor 1989; Orenstein and Brewer 2011). However, there has been very little research on the vocalisations of Mexican, Central American and South American cardinalids (Orenstein and Brewer 2011) and there are no studies considering the effect of body size on the song properties of these species to our knowledge.

We analysed the songs of three species of Neotropical cardinalids: the Glaucous-blue Grosbeak (*Cyanoloxia glaucocaerulea*), the Ultramarine Grosbeak (*Cyanocompsa brissonii*) and the Blue-black Grosbeak (*Cyanocompsa cyanoides*). Both the Ultramarine Grosbeak and Blue-black Grosbeak are polytypic, with subspecies having significant differences in size as well as colour of plumage (Todd 1923; Ridgely and Tudor 1989; Barreira *et al*. 2007). It has also been suggested that songs differ between subspecies (Ridgely and Tudor 1989; Orenstein and Brewer 2011). These taxa thus provide an interesting model to explore the relationship between body size and song frequency within (intraspecific) and between (interspecific) closely related species. We compared the vocalisations of the species to: (1) determine if their songs differ in several spectral or temporal variables, and (2) test the predictions that the subspecies of higher body mass will have lower frequencies in their songs, considering body mass as a proxy of body size. Upon finding considerable variation in temporal parameters, we also test the existence of a relationship of these variables with body mass.

Methods

Study species

The Glaucous-blue Grosbeak is a monotypic species, ~14 cm in total body length and distributed from southern Brazil and eastern Paraguay to north-eastern Argentina (Ridgely and Tudor 1989; Orenstein and Brewer 2011; Fig. 1*a*). The Ultramarine Grosbeak is larger, with a total length of 15–17 cm, and has a broader distribution in South America (Ridgely and Tudor 1989; Fig. 1*b*). The Ultramarine Grosbeak has five subspecies: *C. brissonii brissonii*, *C. b. sterea* and *C. b. argentina* occur in a large area south of the Amazon River, *C. b. minor* is found only in Venezuela, and *C. b. caucae* occupies a small area of Colombia (Clements 2007). The Ultramarine Grosbeak has been shown to be more closely related to the Glaucous-blue Grosbeak than to its congeners, the Blue-black Grosbeak and Blue Bunting (*C. parellina*) (Klicka *et al*. 2007; Bryson *et al*. 2013). The distributional range of the Blue-black Grosbeak extends from Mexico south to Bolivia and Brazil, and does not overlap with that of the Ultramarine Grosbeak (Ridgely and Tudor 1989; Fig. 1*c*). The Blue-black Grosbeak is slightly larger than the Ultramarine Grosbeak, ~17–18.5 cm in total length (Orenstein and Brewer 2011), and has four subspecies: *C. cyanoides concreta*, *C. c. toddi* and *C. c. cyanoides*, which occur in Central America and extreme northern South America, and *C. c. rothschildii*, which is confined to South America (Clements 2007).

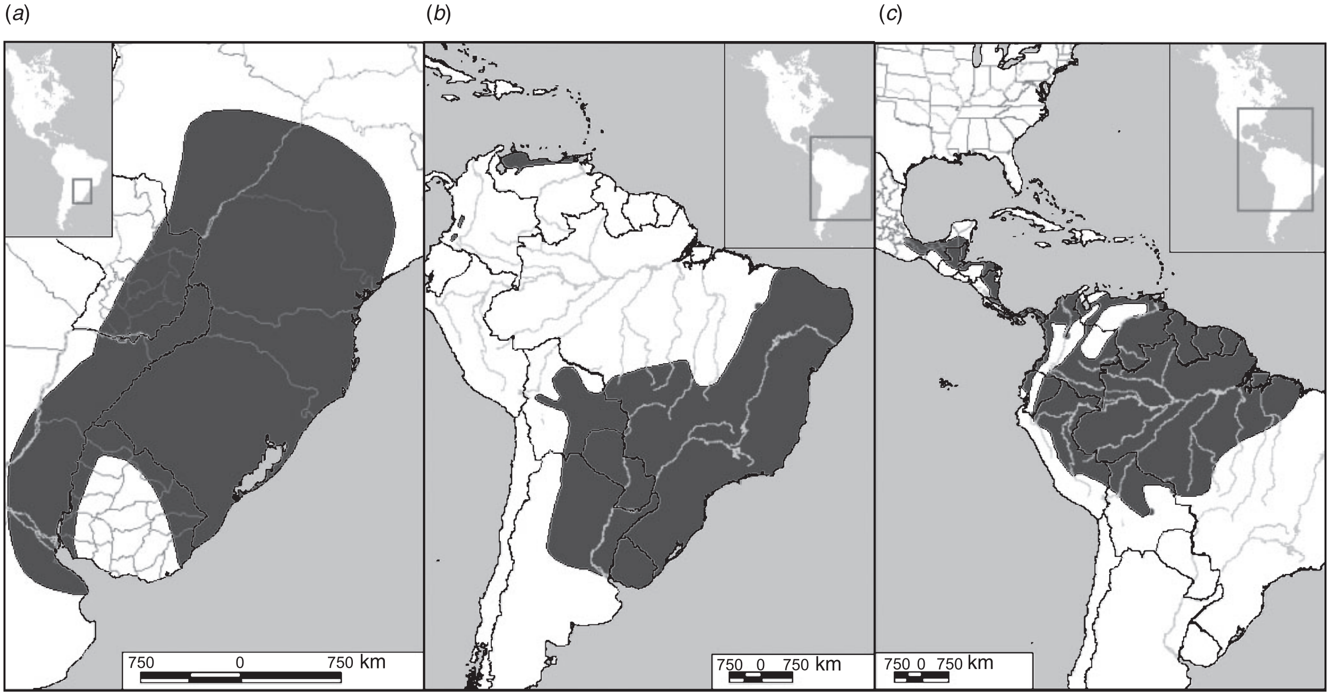

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Fig. 1. Distribution of (*a*) Glaucous-blue Grosbeak; (*b*) Ultramarine Grosbeak; (*c*) Blue-black Grosbeak. Modified from InfoNatura: Animals and Ecosystems of Latin America, version 5.0 (NatureServe: Arlington, VA, see http://www.natureserve.org/infonatura, accessed 4 May 2013).

Song analyses

Recordings used in this study were obtained from the Colección Nacional de Sonidos Naturales of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (Buenos Aires, Argentina), the Macaulay Library of Natural Sounds of the Cornell University (Ithaca, NY, USA), the Bioacoustics Archives and Library of the Florida Museum of Natural History (Gainesville, FL, USA) and the Borror Laboratory of Bioacoustics of the Ohio State University (Columbus, OH, USA). Recordings from several published compilations were analysed (Straneck 1990; Mayer 2000; Krabbe *et al*. 2001; López-Lanús 2009). We used recordings in 'wav' or 'aiff' formats, but those in compressed formats such as 'mp3' were not included.

We analysed recordings of the Glaucous-blue Grosbeak ($n = 9$ songs of different individuals); of three of the five subspecies of Ultramarine Grosbeak: *C. b. minor* ($n = 10$), *C. b. sterea* ($n = 11$) and *C. b. argentina* ($n = 12$); and all four subspecies of Blue-black Grosbeak: *C. c. rothschildii* ($n = 26$), *C. c. cyanoides* ($n = 10$), *C. c. concreta* ($n = 12$) and *C. c. toddi* ($n = 14$). No recordings of subspecies *caucae* of Ultramarine Grosbeak and only mp3 recordings of subspecies *brissonii* of that species were available from the sources we consulted, and so were not included in our study. To make sure each recording was from a different individual and to avoid pseudo-replication, the recordings we selected were made on different dates, locations and recordists, with the exception of cases in which we had explicit evidence the recordings belonged to different individuals.

For each recording, a spectrogram was generated and analysed using the program Raven, ver.1.3 (Bioacoustics Research Program, Cornell Lab of Ornithology, Ithaca, NY, see http://www.birds.cornell.edu/raven), using a 256 fast Fourier transform length with a 50% overlap and a Hamming window of 256 samples. We selected one song per individual and the following temporal variables were measured on the spectrogram (Fig. 2*a*): duration of song (s), note rate (notes s^{-1}), mean duration of notes (s) and mean duration of inter-note interval (s). We defined a note as a continuous trace in the temporal axis of a spectrogram. The note rate was calculated as the number of notes divided by the duration of song. To measure frequency-related variables, we generated an amplitude spectrum for each song, also using a Hamming window but of 512 samples and a 512 fast Fourier transform length. This was done because a larger window provides a better frequency resolution (but poorer time resolution). We measured the emphasised frequency of the song as the frequency of maximum amplitude in the spectrum (Fig. 2*b*). The minimum and maximum frequencies of the song were those at which the amplitude exceeded –30 dB relative to the emphasised frequency (Fig. 2*b*), and the bandwidth was the difference between them. The –30-dB criterion was chosen *a priori*, based on the average quality of the recordings and the signal-to-noise ratio (see Podos 1997; Goodale and Podos 2010).

To describe the vocalisations and look for general differences in song structure we performed a principal components analysis (PCA). This multivariate approach reduces the number of variables from those originally measured to a small number of mutually independent ones (hereafter referred to as principal components (PC)) that account for most of the variation included in the original dataset. We then tested for differences between the studied taxa in their scores for the corresponding PCs with a one-way analysis of variance (ANOVA) followed by Tukey's contrasts. All statistical analyses were performed using Statistica ver.8.0 (StatSoft, Inc., Tulsa, OK, USA).

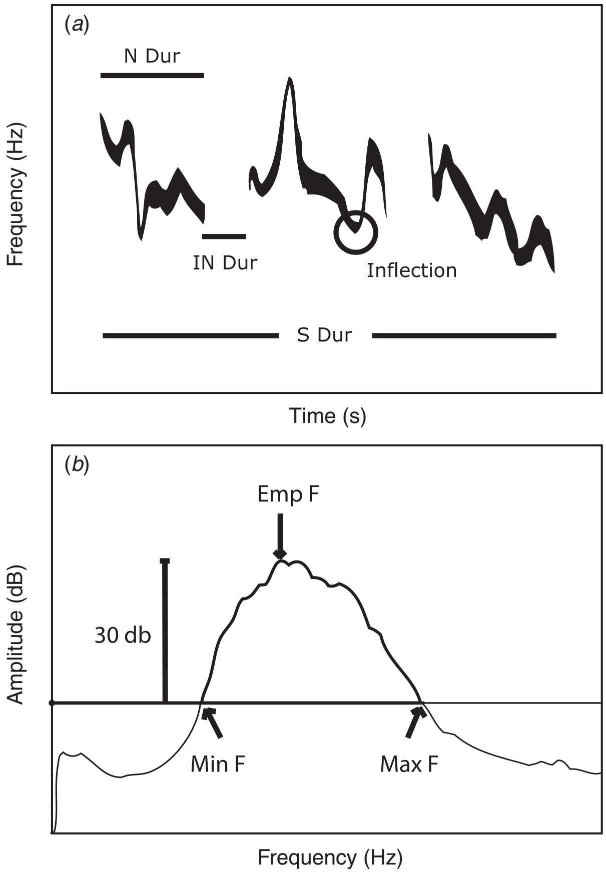

Fig. 2. Measurement of song variables: (*a*) time-related variables, measured on a spectrogram: S Dur, duration of song; N Dur, duration of notes; IN Dur, duration of inter-note interval; and (*b*) frequency-related variables, measured on an amplitude spectrum: Min F, minimum frequency; Max F, maximum frequency; Emp F, emphasised frequency.

Relationship between song traits and body size

To determine if there was a significant relationship between song traits and body mass, we calculated the Pearson correlation coefficient between the mean value of each song variable for each taxa and their mean body mass. Because of their shared phylogenetic history, species cannot be considered as independent points in such comparative studies and a phylogenetic correction is required. The lack of intraspecific phylogenies determining the relationships between subspecies make it impossible to apply such a phylogenetic correction. However, because the studied taxa are closely related (Klicka *et al*. 2007), we considered that the evolutionary differences that could make some groups more similar to each other are not deep enough to bias our analysis significantly.

To calculate the mean body mass of each taxon we used mass data for museum skins of males of Glaucous-blue Grosbeak ($n = 9$), Ultramarine Grosbeak subsp. *sterea* ($n = 8$), Ultramarine

Grosbeak subsp. *argentina* ($n = 7$), Blue-black Grosbeak subsp. *rothschildii* ($n = 21$), Blue-black Grosbeak subsp. *cyanoides* ($n = 8$) and Blue-black Grosbeak subsp. *toddi* ($n = 12$). Date were obtained from the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', the Instituto de Investigación de Recursos Biológicos 'Alexander von Humboldt' (Valle de Leyva, Colombia), the Academy of Natural Sciences (Philadelphia, PA, USA), the National Museum of Natural History, Smithsonian Institution (Washington DC, USA), and the Museum of Vertebrate Zoology, University of California (Berkeley, CA, USA). Data for males of Ultramarine Grosbeak subspecies *minor* ($n = 10$) were obtained from Verea *et al.* (1999). No mass data could be found for Blue-black Grosbeak subspecies *concreta*.

Results

All the taxa we analysed showed complex vocalisations, with a variable number of notes that usually swept through a wide range of frequencies (Fig. 3). These notes usually presented one or more inflections (i.e. a point where the frequency changed from ascending to descending or vice versa; see Fig. 2). Descriptive statistics for the original variables are provided in Table 1. We extracted two principal components with eigenvalues >1 that accounted for >70% of the variation in the original dataset (Table 2). PC1 varied negatively with maximum frequency, emphasised frequency and note rate, and positively with the duration of notes and duration of inter-note interval. PC2 varied positively with bandwidth and negatively with minimum frequency.

The ANOVA performed on PC1 mean scores for each group showed differences at both the interspecific and intraspecific levels (Fig. 4*a*). The song of the Glaucous-blue Grosbeak differed from those of all taxa. This species had the lowest mean score for PC1, with the shortest notes produced at the highest rate, and separated by the shortest interval between notes. It also had the highest maximum and emphasised frequencies. The opposite is

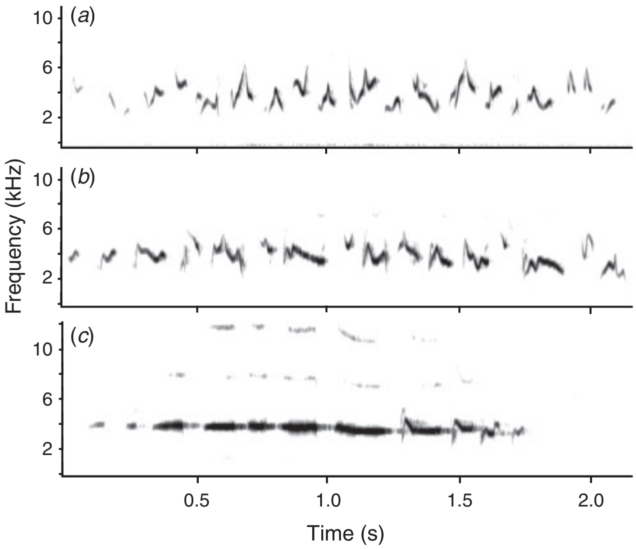

Fig. 3. Representative spectrogram of the song of an individual of each species: (*a*) Glaucous-blue Grosbeak; (*b*) Ultramarine Grosbeak subsp. *minor*; (*c*) Blue-black Grosbeak subsp. *rothschildii.*

Table 1. Body mass (males) and descriptive statistics of the song variables measured for each taxon

Values are means ± s.d. For sources of mass data, see Methods. *n* for body mass and for song variables are different and are given in parentheses

	Glaucous-blue Grosbeak	Ultramarine Grosbeak			Blue-black Grosbeak			
		C. b. minor	*C. b. sterea*	*C. b. argentina*	*C. c. rothschildii*	*C. c. cyanoides*	*C. c. toddi*	*C. c. concreta*
Body mass (g)	17.24 ± 1.34	21.1 ± 2.28	21.47 ± 1.36	24.44 ± 1.83	23.73 ± 2.35	26.46 ± 2.18	29.41 ± 1.82	–
	($n = 9$)	($n = 10$)	($n = 8$)	($n = 7$)	($n = 21$)	($n = 8$)	($n = 12$)	(–)
Song duration (s)	3.03 ± 1.41	2.86 ± 0.65	2.72 ± 0.36	2.62 ± 0.93	2.77 ± 0.66	3.66 ± 0.73	2.82 ± 0.59	2.62 ± 0.29
Note rate (notes s^{-1})	7.52 ± 1.34	5.41 ± 0.37	4.66 ± 0.51	4.69 ± 0.98	4.57 ± 0.56	3.83 ± 0.39	3.32 ± 0.50	3.51 ± 0.55
Note duration (s)	0.08 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.04	0.16 ± 0.02	0.18 ± 0.02	0.20 ± 0.05	0.20 ± 0.04
Inter-note interval duration (s)	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.09 ± 0.01	0.13 ± 0.02	0.11 ± 0.02
Minimum frequency (Hz)	2482 ± 262	2475 ± 251	2404 ± 161	2307 ± 403	2758 ± 383	2265 ± 170	2152 ± 208	2079 ± 392
Maximum frequency (Hz)	6858 ± 632	5664 ± 349	5378 ± 459	5226 ± 661	4553 ± 634	4831 ± 1071	4516 ± 568	4264 ± 629
Bandwidth (Hz)	4376 ± 676	3189 ± 370	2973 ± 509	2920 ± 750	1796 ± 929	2565 ± 1113	2364 ± 578	2185 ± 580
Emphasised frequency (Hz)	4211 ± 419	3600 ± 127	3481 ± 245	3340 ± 229	3449 ± 232	3290 ± 246	3175 ± 300	3139 ± 174
	($n = 9$)	($n = 10$)	($n = 11$)	($n = 12$)	($n = 26$)	($n = 10$)	($n = 14$)	($n = 12$)

Table 2. Loadings of the song variables on the principal components (PC) extracted from the PCA

The song variables that significantly affect the factors (those with loadings >0.7) are identified with an asterisk (*). Factors are unrotated

Song variables	PC1	PC2
Duration of song (s)	–0.07	0.44
Note rate (s^{-1})	–0.92*	–0.14
Duration of notes (s)	0.86*	0.14
Duration of inter-note interval (s)	0.72*	0.31
Minimum frequency (Hz)	–0.33	–0.83*
Maximum frequency (Hz)	–0.82*	0.46
Bandwidth (Hz)	–0.63	0.73*
Emphasised frequency (Hz)	–0.80*	–0.15
Eigenvalue	3.94	1.8
Percentage of explained variance	49	22

true for the Blue-black Grosbeak subspp. *concreta* and *toddi*, which had the highest mean PC1 values. The Blue-black Grosbeak subsp. *rothschildii* differed from two of its conspecifics (*C. c. concreta* and *C. c. toddi*) but had a similar mean value for PC1 to those of Ultramarine Grosbeak subspp. *sterea* and *argentina*. Among Ultramarine Grosbeak subspecies, *C. b. minor* had the lowest mean score for PC1, but there were no differences between *C. b. sterea* and *C. b. argentina*.

Almost all groups had similar mean values for PC2, with the exception of Blue-black Grosbeak subsp. *rothschildii*, which had a significant higher minimum frequency and smaller bandwidth than the other taxa (Fig. 4*b*)

Three frequency-related variables correlated negatively with body mass (see Fig. 5*b–d*): maximum frequency ($r = -0.89$, $P = 0.007$), emphasised frequency ($r = -0.91$, $P = 0.004$) and bandwidth ($r = -0.77$, $P = 0.045$). Three temporal variables also correlated with body mass (Fig. 5*f–h*). Both duration of notes ($r = 0.96$, $P < 0.001$) and duration of inter-note interval ($r = 0.90$, $P = 0.005$) correlated positively with body mass, and note rate correlated negatively ($r = -0.93$, $P = 0.002$). Neither song duration nor minimum frequency correlated with body mass (Fig. 5*a*, *e*)

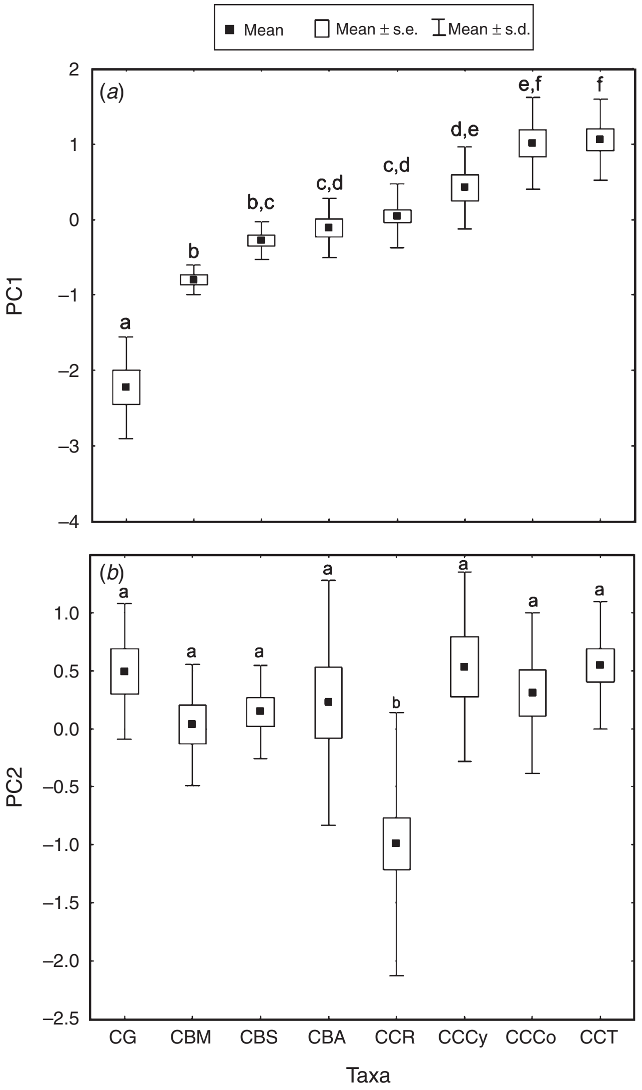

Fig. 4. Scores of the two principal components obtained for each group: (*a*) PC1, and (*b*) PC2. Means ± both s.e. and s.d. are shown. Taxa that differed significantly after Tukey's contrasts are identified with different letters. CG, Glaucous-blue Grosbeak (*Cyanoloxia glaucocaerulea*); CBM, Ultramarine Grosbeak subsp. *minor*; CBS, Ultramarine Grosbeak subsp. *sterea*; CBA, Ultramarine Grosbeak subsp. *argentina*; CCR, Blue-black Grosbeak subsp. *rothschildii*; CCCy, Blue-black Grosbeak subsp. *cyanoides*; CCT, Blue-black Grosbeak subsp. *toddi*; CCCo, Blue-black Grosbeak subsp. *concreta*.

Discussion

The present study constitutes the first objective comparison of the vocalisations of three Neotropical cardinalids: the Glaucous-blue Grosbeak, Ultramarine Grosbeak and Blue-black Grosbeak, and the first attempt to explore the existence of a negative relationship between song frequencies and body size in these closely related species. We found significant differences in songs both within and between species. As expected, we found a negative relationship between three frequency-related variables and body mass. Interestingly, we also found a relationship between temporal variables and this morphological trait, showing that subspecies of lower body mass produced shorter notes at a higher rate, separated by shorter intervals.

We used body mass as a proxy of body size and found that the maximum frequency of the song correlated negatively with it but not the minimum frequency. If the birds are not singing the extreme frequencies they would be able to produce, the correlation with body mass could not be detected. Therefore, one possible explanation is that the taxa here studied are singing frequencies near the maximum possible for them but far from the minimum. Since there is previous evidence in other passerines that females prefer high-frequency rather than low-frequency songs (Dabelsteen and Pedersen 1993; Cardoso *et al*. 2007; Byers 2007), this could be a result of sexual selection pressure on males to sing the highest frequencies possible. In contrast, the size of the syrinx labia is not the only aspect of syrinx morphology that determines song frequencies, and minimum frequency might not be as strongly constrained by labia size as it might be by other factors such as labia shape or labia tension, or both.

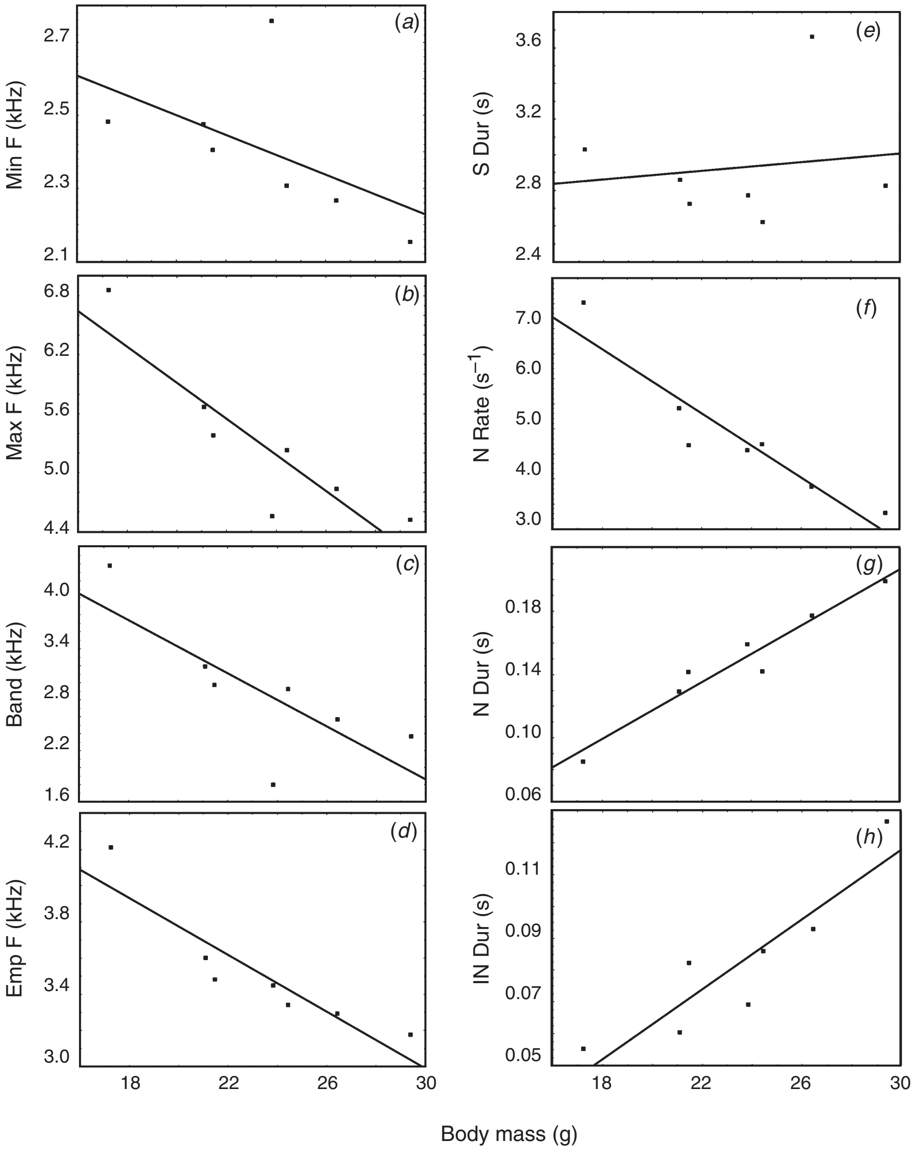

Fig. 5. Scatter plots of song variables *v.* mean body mass, comparing all taxa for which we had body mass data. (*a–d*) Frequency-related variables: Min F, minimum frequency; Max F, maximum frequency; Band, bandwidth; Emp F, emphasised frequency. (*e–h*) Time-related variables: S Dur, duration of song; N rate, note rate; N Dur, duration of notes; IN Dur, duration of inter-note interval.

We also found that the emphasised frequency correlated negatively with body mass. Emphasised frequency is energetically demanding and the constraint imposed by body mass could be even stronger than that imposed on the extreme frequencies. For example, Price *et al.* (2006) measured the frequency with the highest amplitude at regular intervals throughout a song to determine the lowest and highest peak frequencies of the song for each of 102 icterid taxa, and found that the lowest peak frequency correlated negatively with body size in this group. They measured these variables instead of the maximum and minimum frequencies because they considered them to be a more physiologically meaningful measure of performance than the extreme frequencies. Previously, Bowman (1979) had found no correlation between body mass and the maximum and minimum frequencies of the song when comparing Darwin's Finches (*Certhidea* sp., *Geospiza* spp., *Pinaroloxias* sp., *Camarhynchus* spp., *Cactospiza* spp. and *Platyspiza* spp.). However, he found that the frequency at which peak energy of song occurred showed a negative correlation with body mass.

Three of the temporal variables we measured also correlated with body mass: note rate, duration of notes and duration of internote interval. Heavier taxa had longer notes, produced at lower rates and separated by longer intervals. Variation in temporal song traits among species has been traditionally related to the effect of the habitat on song evolution (Morton 1975; Ryan and Brenowitz 1985; Wiley 1991; Tubaro and Lijtmaer 2006). However, morphological traits can also influence the temporal structure of avian song. Given the importance of the resonating properties of the suprasyringeal structures to produce proper vocalisations (Nowicki 1987; Hoese *et al*. 2000) birds open and close their bills when singing to change the total length of their vocal tract and can thus track the fundamental frequency produced by the syrinx (Nowicki 1987; Westneat *et al*. 1993; Podos *et al*. 2004). However, there is a limit to how rapidly an individual can change its bill-gape, and such a limit would be highly related to the size of the bill (Nowicki *et al*. 1992; Podos 1997; 2001). Consequently, the size of the bill could influence the temporal structure of songs, particularly in birds producing difficult songs, such as trills (series of rapid frequency-modulated notes, repeated in a succession at a constant tempo; see Podos 2001; Derryberry 2009). The species studied here do not use trills in their songs, but the notes they use can be considered difficult because they are usually frequency-modulated, with one or more inflections per note. The taxa studied here have stout, heavy bills that could affect the temporal structure of their vocalisations by limiting the speed of movements of the bill that are necessary to track the fundamental frequency of the note. Bill-size in these species correlates with body size and mass (N. C. García, pers. obs.), which may explain why we found that heavier taxa produced notes at lower rates, separated by longer intervals. It would be interesting in the future to perform a more detailed study to analyse if there is an effect of bill-size on song structure, independent of the effect of body mass we found.

In the present study, body mass was used as an indicator of body size, which could be problematic because these two parameters are not necessarily directly correlated. Some authors consider that body size is better represented as a combination of several body measurements, such as length of skull, keel, wing-chord and tarsus, and mass (Rising and Somers 1989; Freeman and Jackson 1990; Piersma and Davidson 1991). However, it is not always possible to obtain such measurements (particularly of skeletal elements), and body mass is the preferred univariate measure of avian body size (Freeman and Jackson 1990). Additionally, many previous studies that analysed the effect of body size on song properties have used body mass as an indicator of body size (Wallschläger 1980; Tubaro and Mahler 1998; Bertelli and Tubaro 2002; Seddon 2005), which makes this parameter useful for the comparison of results of different studies.

Ideally, body mass and song data from the same bird should be used for the exploration of the relationship between these variables (Cardoso *et al*. 2008; Martin *et al*. 2011). However, it is complex and difficult to do so, especially when trying to analyse differences between groups that are distributed over broad geographical ranges, such as the taxa considered here. Sound databases and libraries, where recorded birds are almost never captured and measured, are the most suitable data source for a comprehensive analysis. Therefore, most of the previous literature on this subject used recordings and mass data derived from different specimens, as we did here (Wallschläger 1980; Tubaro and Mahler 1998; Bertelli and Tubaro 2002; Seddon 2005). This could lead to estimation errors, given the variation in mass between individuals, seasons and collection years. The most likely effect of these uncontrolled sources of variation is a higher chance of not detecting any existing relationship between frequencies and mass rather than creating a spurious one. Therefore, we are confident that the highly significant relationships we found are another example of the strong influence of body size in song evolution.

To sum up, even though differences among closely related groups may be small, our results suggest that the negative relationship between body mass and song frequencies found in a large number of bird species (Wallschläger 1980; Ryan and Brenowitz 1985; Tubaro and Mahler 1998; Bertelli and Tubaro 2002; Seddon 2005) may also be found among closely related groups, such as sister species or subspecies. We also found an association of the temporal structure of the song with body mass, which highlights the importance of considering the influence of this factor in time-related variables as well when studying song evolution.

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