PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

INFLUÊNCIA DO BICO NA ESTRUTURA DO CANTO EM PAPA-CAPINS NEOTROPICAIS (THRAUPIDAE: *SPOROPHILA*)

Natália Simoni Porzio

DISSERTAÇÃO DE MESTRADO

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Orientadora: Dr. Carla Suertegaray Fontana

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Sumário

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Resumo

A principal forma de comunicação das aves é por meio de sinais acústicos, é através deles que elas atraem parceiros, defendem territórios e se reconhecem. Com isso, o canto é extremamente importante no processo de especiação, em particular nas aves Oscines, um grupo que tem amplos tipos de canto, singulares a cada espécie. Sabe-se que o trato vocal atua como modulador na estrutura do som; características do bico podem atuar como fatores limitantes influenciando a taxa de notas, largura de banda e frequências, no qual bicos maiores produzem sons com menor taxa de notas e com frequências mais restritas. O nosso trabalho teve dois objetivos relacionados a um grupo de aves Oscines (*Sporophila*), o primeiro (capítulo I) foi analisar a evolução do volume do bico e da estrutura do canto para verificar a existência de limitações e coevolução entre os mesmos. No segundo (capítulo II) testamos a relação entre volume do bico e a capacidade de mordida com a estrutura do canto de três espécies simpátricas de *Sporophila* spp., além de verificar como estas aves organizam seus cantos para evitar a sobreposição deles no ambiente. No capítulo I analisamos cantos territoriais representando 11 espécies de *Sporophila*. Encontramos que a largura de banda e máxima frequência tiveram relação negativa com o volume do bico; além disso, a frequência mínima apresentou uma tendência para o mesmo padrão. O volume do bico, taxa de notas e frequência mínima demonstraram ter sinal filogenético, sugerindo que espécies proximamente relacionadas apresentem essas variáveis mais similares. Nossos resultados mostram que o volume do bico atua como limitador na estrutura do canto além de indicar que esse fator pode coevoluir com o canto destas aves, corroborando a literatura. Entretanto, a frequência dominante, taxa de notas e duração do canto não apresentaram limitação pelo volume do bico, o que sugere que ainda existem muitas informações sobre o tema para serem descobertas. Assim é interessante que se teste a influência filogenética, além dos caracteres morfológicos sobre as características do canto em aves Oscines. No segundo capítulo nós selecionamos *S. beltoni*, *S. caerulescens* e *S. hypoxantha* como modelos de espécie grande, média e pequena respectivamente. Estes três táxons costumam coexistir e se reproduzir nas mesmas áreas no sul do Brasil e foram amostrados concomitantemente no nordeste do Rio Grande do Sul. Machos adultos foram observados e gravados e após foram capturados, tiveram seus bicos medidos e passaram pelo teste de pressão de mordida em campo. Nós encontramos que *S. beltoni* e *S. hypoxantha* são as espécies que mais divergem em parâmetros acústicos, sendo que a primeira apresenta bico mais volumoso dentre as três espécies. A maior relação entre força/tamanho do bico e o canto foi observada em *Sporophila caerulescens* na qual a largura de banda e frequência máxima/mínima do canto apresentaram relação positiva com a força e o tamanho do bico, evidenciando que este caráter não limita o som deste táxon ao contrário do que outros estudos têm mostrado. Referente à ocupação do espaço acústico, as maiores diferenças encontradas foram relacionadas às variáveis de tempo do canto e taxa de notas. Cogita-se que estes podem ser alguns dos fatores usados para evitar a sobreposição dos cantos na natureza. Em termos gerais nosso estudo mostra que a limitação do canto por caracteres do bico não é uma regra geral em Oscines embora exista uma relação negativa entre variações no canto e tamanho do bico quando mais espécies sçao adicionadas ao modelo. Com esses resultados nós acreditamos que é interessante seguir pesquisando sobre os fatores “limitadores do canto” já que não existe um padrão generalizado para aves canoras. É importante conhecer como as mudanças na morfologia de todo o trato vocal podem alterar a estrutura do som das aves e como caracteres morfológicos e acústicos evoluíram através da sua história filogenética, já que este entendimento é essencial para melhor compreender o processo de especiação destes grupos.

Abstract

Acoustical signals are the main form in which birds communicate, and they are used to attract mates, defend territories, alarm others from predators and recognize each other, being than an important feature on speciation process. Then, song is extremely important, especially in Oscines, a group of birds that have a widely types of songs, singular for each species. Many studies have been shown that the vocal tract act as a constrict factor on song structure, besides body size. Beak gape, size and bite force are known as a limiting factors of note rate, frequency bandwidth and maximum/minimum frequencies, in which larger beaks produces narrow sounds with lower note rates. Our study had two objectives related of Oscine birds (of the genus *Sporophila*): the first one (chapter I) was to analyze the evolution of beak volume and song structure and verify the existence of song constriction and coevolution between them. The other objective (discussed in chapter II) was to test the relation between beak volumes and bite force with the song structure of three sympatric species of *Sporophila*, and try to infer how they organize their songs to avoid the acoustic overlap due their coexistence. In the chapter I we analyze territorial songs of 47 males from 11 species of *Sporophila*, representatives of Neotropical Oscines (Thraupidae). We found that frequency bandwidth and maximum frequency had a negative relation with beak volume, besides that, minimum frequency showed a trend for the same pattern. Beak volume, note rate and minimum frequency demonstrated to have a phylogenetic signal, which suggest that closely related species had beak morphology aand song structure more similar then lesser related species. Our results corroborate literature, in which beak volume acts as a limiter for song structure and also that this character can coevolve with birdsong. However, other variables did not present a constriction by the beak volume, what suggest that there is much to discover about this relation in songbirds yet. It is interesting to test the phylogenetic influence besides morphological characters, since this feature can be influencing song structure more than song constrainers. In chapter II, we selected *S. beltoni*, *S. caerulescens* and *S. hypoxantha* as a model of large, medium and small species respectively. These three species used to occur in the same habitat where they breed in northern of Rio Grande do Sul, Brazil. Adult that were observed and recorded were captured, had they beak measured and pass though bite force test. We found that *S. beltoni* and *S. hypoxantha* had more differences among song parameters, in which the first one presents the larger beak among the three species evaluated, and the second the smallest beak. The highest relation between strength/beak size and song was observed in *S. caerulescens* in which frequency bandwidth and minimum/maximum frequencies presented a positive relationship with bite force and beak size, showing that this feature do not limit song on this taxa against that other studies have shown. Concerning to the use of acoustical space, the greatest differences found were related with note rate and song duration. These factors should be some features used to avoid the song overlap on nature. In general terms our study shows that the limitation of sound per characters of the beak it is not a rule in Oscines, although there is a negative relationship between song variation and beak size when more species are add to the model. With these results we belive that it is important to know how changes in morphology of all vocal tract can affect song structure and how morphologic and acoustic features had evolved through their phylogenetic history, because understand this is essential to better comprise the speciation process among these groups.

Apresentação

Sinais acústicos são extremamente importantes para a comunicação das aves, é através deles que os indivíduos defendem seus territórios, atraem parceiros, avisam sobre a presença de predadores e realizam o reconhecimento intraespecífico (Marler et al. 2004; Catchpole e Slater 2008). Os sinais acústicos são essenciais na evolução e divergência das aves, pois eles atuam como barreira pré-zigótica no processo de especiação (Catchpole e Slater 1995; Price 2008). A ordem Passeriformes é dividida em *Oscines* (aves canoras) e *Suboscines*, no qual o primeiro grupo apresenta uma musculatura singular associada ao órgão vocal, a siringe (Ames 1971). A siringe é a principal estrutura que gera o som e atua similarmente ao sistema observado em humanos, onde a passagem de ar por membranas internas desse órgão resultam na produção do som (Greenwalt 1968; Larsen e Goller 1999; Larsen e Goller 2002; Suthers e Zoolinger 2004). Somente os *Oscines* podem produzir cantos complexos, uma forma de vocalização que apresenta variação de notas e frequências, os quais são possíveis devido à presença de alguns pares de músculos associados à siringe, característica que é utilizada para distinção de táxons (Catchpole e Slater 2008).

O trato vocal também é composto pela traqueia, laringe e bico, estruturas que têm influência no resultado final do som produzido em aves (Nowicki e Marler 1988). Além disso, o tamanho corpóreo pode limitar as frequências produzidas nos cantos, demonstrando uma relação negativa entre o tamanho do indivíduo e as frequências mais elevadas (Martin et al. 2011; Mason e Burns 2015). Aves maiores podem apresentar siringes proporcionais e tratos vocais mais longos, o que resulta em sons de menor frequência (Hoese et al. 2000). Dentre os caracteres que limitam o canto, o bico é o mais estudado devido a sua variação na forma e tamanho (Hoese et al. 2000; Ballentine 2006; Herrel et al. 2009; Ohms et al. 2010; Derryberry et al. 2012). A abertura do bico está relacionada com a musculatura associada à mandíbula e maxila, além da capacidade de mordida, com isso a velocidade que o animal realiza este movimento interfere na estrutura do canto (Herrel et al. 2005). A ressonância do trato vocal é modificada quando o indivíduo abre e fecha o bico, no qual aves com ampla abertura tendem a produzir sons de maior frequência e vice versa (Bellentine 2006). O padrão observado para a diversificação do formato e força do bico é geralmente um subproduto de adaptações alimentares, como o observado no gênero *Geospiza* nas ilhas de

Galápagos (Grant e Grant 1985; Herrel et al. 2005; Westneat et al. 2005; Ballentine 2006; Genbrugge et al. 2011).

Os tentilhões de Darwin apresentam ampla variação de formato e tamanho do bico devido ao processo de especiação, além disso, estas aves têm uma filogenia bem conhecida, sendo um grupo monofilético (Grant et al. 1985; Burns et al. 2014). Estas características fazem dos tentilhões um ótimo grupo para se testar a relação entre som, caracteres morfológicos limitadores e a evolução deles dentro deste grupo. Com essa informação é possível avaliar como a estrutura do som é influenciada pela filogenética e os caracteres morfológicos. Estudos com estas aves revelam que a variação do bico está relacionada com a capacidade de mordida e a estrutura do som, no qual aves com bicos maiores e mais fortes, adaptados para quebrar sementes rígidas, produzem sons de frequências mais limitadas e menor taxa de repetição de notas, diferente de aves com bicos menores, adaptados para comer insetos, e têm menor limitação nestes parâmetros do canto (Podos 2001; Podos e Nowicki 2004; Herrel et al. 2009).

Um estudo com furnarídeos mostrou que espécies com bicos mais longos produzem cantos com menor número de notas por tempo além de ter cantos com largura de banda mais estreita, quando comparados às espécies de bicos mais curtos (Derryberry 2012). Eles também analisaram a evolução vocal em Dendrocolaptinae e encontraram uma correlação entre a morfologia do bico e a estrutura do som, onde neste grupo a diversificação e a evolução do som teriam evoluído sob a influência do tamanho do bico. Os tentilhões de Darwin apresentam a mesma correlação entre a morfologia do bico e a evolução do som, demonstrando a similaridade entre a limitação do canto em grupos de *Oscines* e *Suboscines* (Podos 2001).

Dentre as aves *Oscines* Neotropicais os papa-capins são um grupo extremamente diverso (Thraupidae: *Sporophila*) que contém mais de 30 espécies que ocorrem desde o norte da América Central até a América do Sul (Rising et al. 2011). Essas aves habitam áreas abertas ou semiabertas e se alimentam principalmente de sementes de Poaceae, Cyperacea ou gramíneas (Sick 1997). Papa-capins são aves pequenas podendo chegar até 16 g de massa corporal e 15 cm de comprimento total, os machos apresentam uma ampla variação na coloração da plumagem enquanto que as fêmeas apresentam cor oliva ou marrom (Sick 1997; Ridgely e Tudor 2009; Rising et al. 2011). Sporophilinae é um grupo monofilético e apresenta um clado que divergiu recentemente, o dos caboclinhos, um grupo com baixa diferenciação genética (Litjmaer et al. 2004; Burns et al. 2014). Apesar da existência de espécies com baixa separação genotípica, dados acústicos deste gênero mostram a grande variação no canto dos machos (Campagna et al. 2010; Areta e Repenning 2011).

O canto em *Oscines* atua como uma barreira reprodutiva, sendo assim um fator determinante no processo de especiação (Grant e Grant 2009). Diferenças na morfologia, canto e estrutura genética das aves podem ser resultado de especiação alopátrica ou, num cenário sem barreiras físicas em que há segregação no uso de habitat, uma especiação simpátrica (Marler e Peters 1982; Sick 1997; Catchpole e Slater 2008; Slabbekkorn e Smith 2002). A variação no canto pode ser resultado de divergência genética (Marler e Peters 1982; Catchpole e Slater 2008), alterações resultantes do aprendizado vocal ((Podos et al. 2004, Catchpole e Slater 2008) ou por ambos os processos. A morfologia do bico como limitante na performance vocal é bem conhecida em um grupo particular, nos tentilhões de Darwin, com isso é importante que se realizem mais estudos sobre esta relação mas em outros grupos de aves para verificar se este padrão é generalizado em *Oscines* ou não.

Sporophila é um gênero interessante para ser estudado já que congrega species com leve diferenciação em tamanho corpóreo e ampla diversidade no tamanho dos bicos, além deste grupo apresentar uma enorme variedade na estrutura do canto dos machos. O gênero *Sporpohila* tem a filogenia bem conhecida o que nos permite avaliar a influência do parentesco entre as espécies na evolução da estrutura do canto e no tamanho do bico. Neste trabalho testamos a relação entre o volume do bico e a estrutura do canto territorial em papa-capins Neotropicais. Nossos resultados foram divididos em dois capítulos, o primeiro é intitulado "Evolution of beak size and song constraint in Neotropical Seedeaters (Thraupidae: *Sporophila*)", o qual nós realizamos a análise filogenética em adição aos dados morfológicos do bico e aos parâmetros do som para verificar a limitação no canto produzido por espécies de *Sporophila*. Com esta análise podemos avaliar se as diferenças apresentadas são relacionadas apenas com a morfologia do bico ou se apresentam influência filogenética. No segundo capítulo, intitulado "Does beak volume and bite force influence song structure of sympatric species of Neotropical seedeaters finches (Thraupidae: *Sporophila*)?" testamos o efeito do volume do bico e capacidade de mordida sobre a estrutura do canto em três species simpátricas (*Sporophila beltoni*, *Sporophila caerulescens* e *Sporophila hypoxantha*), e como estas espécies segregam o espaço acústico o qual elas coexistem. Nós pretendemos enviar ambos os artigos para revisão profissional de inglês para submissão às revistas científicas. O capítulo I será enviado para a revista Evolution e o capítulo II para a revista Animal Behaviour.

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CAPÍTULO I

Revista de submissão: Evolution – Conceito CAPES: A1

Evolution of beak size and song constraint in Neotropical Seedeaters (Thraupidae: *Sporophila*)

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Abstract

Birdsong diversity is a result of the influence of many traits on songs. Acoustical parameters can be constraint by morphological traits, as body and beak size, bite force, or by phylogenetic relationships. Understand mechanisms that acts as magic traits are important to clarify how these changes can catalyses speciation and how they evolve over the groups. Within Neotropical passerines there is an extremely diverse group of seedeaters, the *Sporophila* (Thraupidae) which presents a widely variation in beak size despite conservative shape. We analyzed the evolution of beak volume and song constraint based on the differences of their morphological feature in *Sporophila* species. We performed PGLS analyses which were evaluated the relationship of seven song parameters of territorial songs from 47 adult males of eleven species of *Sporophila* and beak volume. We found a negative relation between beak size and two song parameters, frequency bandwidth and maximum frequency. Besides that, the minimum frequency shows a tendency for a negative relation either. Body mass and beak volume demonstrated a positive relation between them, besides that, two characters had a positive phylogenetic signal, beak volume and note rate. Our results corroborate prediction that beak size can act as a limiter of song structure in Oscine birds, since the frequency bandwidth and the maximum/minimum frequencies were influenced by beak size. However, as we found, many parameters of song can be a consequence of species' phylogenetic history. Phylogeny may promote that more closely related species have more similar features, as beak volume, note rate and minimum frequency as notted here. Concerning this subject, is important to emphatize researches on birdsong and its limiters, especially in Oscines, including phylogenetic analysis that allow us to evaluate how these variables act on song structure. In *Sporophila* spp. morphoogical traits and phylogeny had influence on their song structure.

Keywords: magic traits, diversification, birdsong, acoustic communication, oscine.

INTRODUCTION

In birds acoustic signals are used for mate choice, territory defense and group communication being thus a determinant factor for life history of these animals (Catchpole and Slater 2008). Song is one of the most important traits that have potential and capacity to promote speciation process in birds (Catchpole and Slater 2008; Price 2008). Genetic and morphologic traits can be very similar between some taxa, thereby acoustic signals end up acting as the isolating mechanism that allows the differentiation for these species, avoiding their recognition and interbreeding (Edwards et al. 2005).

Birds have basically two types of vocalizations: calls, a simplest form, and song, a complex one (Catchpole and Slater 2008). Oscines are a specialized group that present singular pairs of muscles associated at vocal organ, the syrinx (Ames 1971; Suthers and Zoolinger 2004). This feature allows them to produce a wide range of songs with different frequencies, durations and note shapes, thus being a mean of communication extremely complex mainly produced by males (Ames 1971; Catchpole and Slater 2008). The sound is generated by the syrinx but until it emission by the beak there is a way through the vocal tract, which comprehends the trachea, larynx and beak (Nowicki and Marler 1988). During this journey song pass through changes, and the result of all this constraints and interferences (features that act on song structure) are seen when a bird sings (Nowicki 1987; Hoese et al. 2000; Ohms et al. 2010).

The variation in song structure is also associated with other aspects, as beak size and form, body size, phylogenetic relationship among species, mimic capacity and learn quality (Ryan and Brenowitz 1985; Podos 2001; Podos and Nowicki 2004; Ballentine 2006; Martin et al. 2011; Derryberry et al. 2012). Besides that, female preferences can influence male’s selection, which affects consequently in future generations, since many Oscine birds learn their repertories from their parents, especially from the males (Nowicki and Marler 1988; Ballentine et al. 2004; Catchpole and Slater 2008; Podos and Moseley 2009). All this mechanisms that can promote song diversity may increase divergence between populations and with this, catalyses speciation (Podos et al. 2012). These structures have to be investigated especially among Oscine birds, since they present a higher rate of evolution due to cultural behavior of learn songs when compared to birds that have innate songs.

Bite force, beak gape, size and shape are known as motor constraints of song in many bird species modulating the acoustic parameters (Hoese et al. 2000; Ballentine 2006; Herrel et al. 2009; Ohms et al. 2010; Derryberry et al. 2012). These variations in the morphology of the beak are, mostly, a byproduct of food adaptation, which can cause changes in the resonance filter of vocal tract and may results in different structures of songs produced (Hoese et al. 2000). This scenario when a structure evolves or undergoes changes and this shift promotes variation in other characters is known as ‘magic trait’. Beak size and shape are considered ‘magic traits’ when changes in beak morphologies resulting from a food adaptation, for example, affect the singing capability (Servedio et al. 2011; Podos et al. 2013).

Darwin’s finches are well studied group that demonstrates how beak size and force can act as a ‘magic trait’ for bird songs (Podos 2001; Podos and Nowicki 2004; Herrel et al. 2009). Finch’s populations from Galápagos Islands adapted their beaks according to food resources in addition to individual competition and as a byproduct of this morphological modification, song structure of these birds also pass through changes due this magic trait, beak size and shape variation (Grant et al. 1976). An individual that has larger beaks produces songs with lower frequencies, lower note rate and narrow frequency bandwidth, as opposite to smallest ones (Podos 2001; Derryberry 2012). Larger beaks and stronger bites may present a bigger muscles associated with them, feature developed for crush hard seeds, for example, which results in a greater constrict of beak movements, unlike birds that feed on grass or insects that present a thinner muscles what does not limit their movements of the beak (Herrel et al. 2005; Genbrugge et al. 2011).

Many studies have shown beak features *vs* sound present a negative relation, that is, the larger the beak and the bite capacity the lower is the frequency and the note rate produced (Hoese et al. 2000; Podos 2001; Derryberry et al. 2012). Birds with larger beaks and strong bites may have more constraint to move the jaws and as a consequence of this limitation, the song has lower frequencies and the note rate is decreased (Herrel et al. 2009). Body size is also responsible for song constraint and has the same negative relation with song parameters, in which heavier birds may present lower frequencies (Martin et al. 2011; Mason and Burns 2015).

In addition to song constraint evaluations, other variables should be considered, as the influence of evolutionary relationship in song parameters and morphological characters (Podos 2001; Derryberry et al. 2012; Riede et al. 2016). Closely related species are expected to share or present more similar traits such as: acoustic, genetic and morphologic features (Paradis 2012). Darwin's finches (Emberizidae: *Geospiza*) have a well-known phylogeny being a monophyletic group, which permits evaluate how song structure (e.g. frequencies, note rate and song duration) is constrained by phylogenetic and morphologic characters (Burns et al. 2014). For this genus Podos and Nowicki (2004) described that song evolve with constraints by beak morphology. However, these birds have passed through a very particular process of speciation in island with an availability of assorted foods that resulted in a widely variation of beak form adaptation (Grant et al. 1976; Grant and Grant 1979; Grant et al. 1985). It is necessary to investigate whether this pattern occur in other songbirds that have morphologic divergence and a known phylogenetic relationship to test this prediction.

Seedeaters of genus *Sporophila* include about 34 species of the Neotropics that occurs from Central to South America (Rising et al. 2011). They have a variation in body mass (e.g. *Sporophila minuta*, 7.9 g and *Sporophila leucoptera*, up to 16 g) and beak size, plumage and elaborate male's vocalizations (Meyer de Shauensee 1952; Rising et al. 2011). In this genus, mainly males have differences in plumage and most of the females have olive or brown plumage being very difficult to differentiate them (Ridgely and Tudor 1989; Sick 1997; Rising et al. 2011). A strong characteristic besides male's coloration in this group is the territorial song of them, which is extremely variable between all species.

Evolutionary changes in beak morphology tend to have an impact on sound structure. However, many characteristics observed in specie's song can be a reflection of its phylogenetic history (Podos 2001; Derryberry et al. 2012; Riede et al. 2016). We choose work with the genus *Sporophila* because it presents a great diversity in song structure and beak size, besides having a recent phylogeny published, what make possible evaluate this influence on song evolution (Burns et al. 2014). Analyzing both variables make possible to detect how constraints had evolved through a group and how they are related. For that, we tested if beak volume acts as a magic trait for song scructure testing the prediction that beak size and structural song parameters (e.g. peak/maximum/minimum frequencies, note rate, frequency bandwidth, number of notes) have a negative relation. Besides that we verify if exist phylogenetic influence on song parameters of *Sporophila* species.

MATERIALS AND METHODOS

Group of Study

We analyzed eleven species of seedeaters inserted in a parametric tree (Fig. 1) generated with software Fig.tree (v.1.4.3) according to Burns et al. (2014) topology which they used molecular data. Among the representatives we include *Sporophila lineola*, as a sister species with two others clades. In the first group were included specimens of capuchinos (*Sporophila minuta*, *S. melanogaster*, *S. pileata* and *S. hypoxantha*) which is a group that had recently diverged (Campagna et al. 2010; Campagna et al. 2013; Burns et al. 2014). The other one has specimens of *Sporophila nigricollis*, *S. caerulescens*, *S. collaris*, *S. leucoptera*, *S. beltoni* and *S. plumbea*. One particular case is the insertion of *S. beltoni* as a pautative sister species of *S. plumea*. The first one was described recently and it is not present in any phylogeny, but is empirical known it relation with *S. plumbea* (Repenning and Fontana 2013). With these eleven species we obtained a representative sample o species that have a widely differences in beak volume and strong variation in song structure. Such choice enabled us to test the constraint relationship between beak volume and song variation on the evolution of Neotropical Seedeaters.

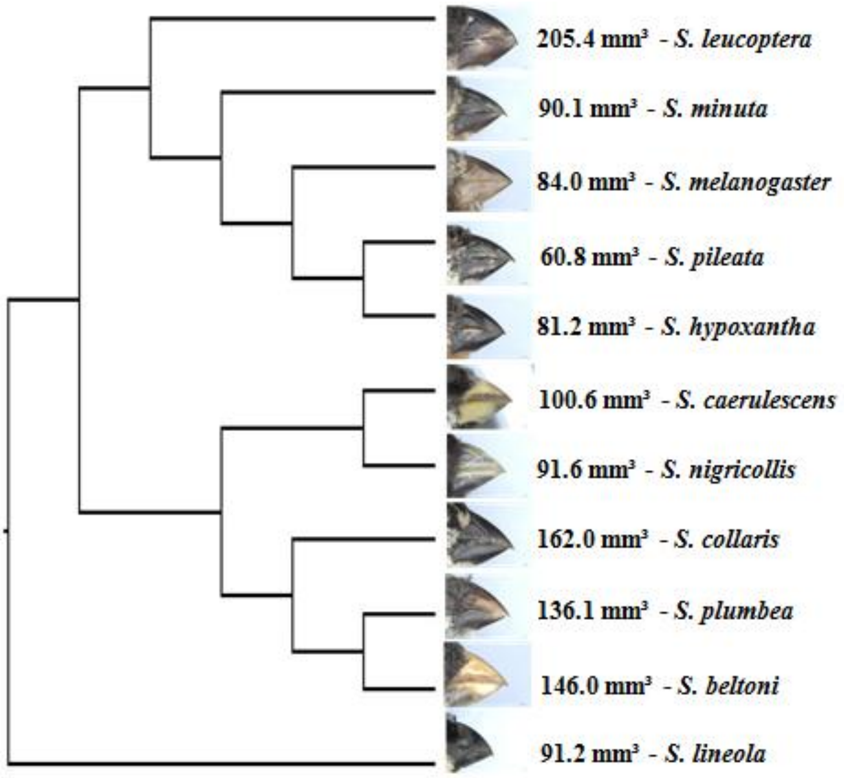

Figure 1 Phylogenetic tree of eleven Neotropical Seedeaters with the inclusion of morphological characters of beak volume based on Burns et al. (2014) topology. On the right there is beak volume and the reference species.

Morphologic and Song Analysis

The whole song and morphological data used were from individuals available at Museum of Science and Technology of PUCRS, Porto Alegre, Brazil. All the specimens available in the collection that we used had a skin complete with the weigh data and song recorded in field. We evaluated 47 adult males from 11 species, being them: *S. melanogaster* (n=2), *S. collaris* (n=4), *S. leucoptera* (n=6), *S. nigricollis* (n=3), *S. pileata* (n=4), *S. minuta* (n=2), *S. lineola* (n=1), *S. plumbea* (n=2), *S. beltoni* (n=10), *S. hypoxantha* (n=6) and *S. caerulescens* (n=6). Beak measurements were made using a metallic caliper rule (scale 0.1 mm). We calculated beak volume using the cone volume formula [$v = (\pi.r^2.h)/3$] what best characterizes the beak shape of the specimens, where r = beak height/2 and h = beak length (exposed culmen). For evaluate song structure we check three territorial songs (phrases) for each bird and, after that, we calculated the mean of the parameters for statistical analyzes. We selected seven song variables including four by frequency (peak, min, max and BW) and three by time (duration, number of notes and note rate). All song analysis were performed using Raven Pro 1.5 software. To calculate the frequency bandwidth we utilized amplitude spectra and we determined it as the difference between the maximum and minimum frequencies, using a -24 dB relative to the peak amplitude frequency in song (Podos 1997; Podos 2001). The spectrograms configuration were a Hann window, DFT size=1024, overlap=75% and bandwidth = 270 Hz. We used an oscillogram to measure song duration, which we could delineate this variable using an on-screen cursor for select each phrase, number of notes and, consequently, note rate (note/s: number of notes per time). Song records used in our study were made with a Teac Tascam DR 680 or Nagra LB, with external microphone Sennheiser ME62 associated to a Telinga Parabola (21.5 in/6.1 in). Songs will be deposit on the Macaulay Library of Natural Sounds, Cornell Lab of Ornithology (Ithaca, NY).

Statistical Analysis

To verify the relation between beak volume and song constriction on the seedeaters we performed a Phylogenetic Generalized Least Squares (PGLS) analysis. We also evaluated the relation with body mass and beak volume using the same analysis. With this test we can compare song and morphological data among species considering their plylogenetic relationships. PGLS assumes that closely related species may have more similar features due to their shared ancestry and incorporate this information in the regression equation. The tree used in our analysis had a Nexus format and had the same topology of Burns et al. (2014), with the inclusion of *S. beltoni* as closely related to *S. plumbea* (Repenning and Fontata in prep.) and we set all the branch length as equivalents (Purvis et al. 1994). The existence of coevolution relationship between beak volume and song constraint was tested for each song parameter, as dependent variable, and beak volume, as independent one. We also verify the phylogenetic signal for each song parameter and beak volume, what demonstrates the tendency of closely species present or not similar features (Paradis 2014). All statistical analysis were performed using R (R Core Team 2016) with the packages 'nlme' and 'ape' for PGLS test and 'ggplot2' to make the graphs. Differences were significant when $P < 0.05$.

RESULTS

We found a negative relation between maximum frequency ($P < 0.05$, *Slope* = 48.71) and frequency bandwidth ($P < 0.05$, *Slope* = 35.41) with the beak volume (Fig. 2). Additionally minimum frequency did not demonstrated statistically significant result, despite this it had a weak tendency ($P = 0.18$, *Slope* = 12.66) for the same pattern (Fig. 2). The other parameters measured did not present statistically significant results or tendencies: number of notes ($P = 0.46$, *Slope* < 0.00); note rate ($P = 0.79$, *Slope* = -0.004); song duration ($P = 0.98$, *Slope* = 35.41) and peak frequency ($P = 0.90$, *Slope* = -0.013).

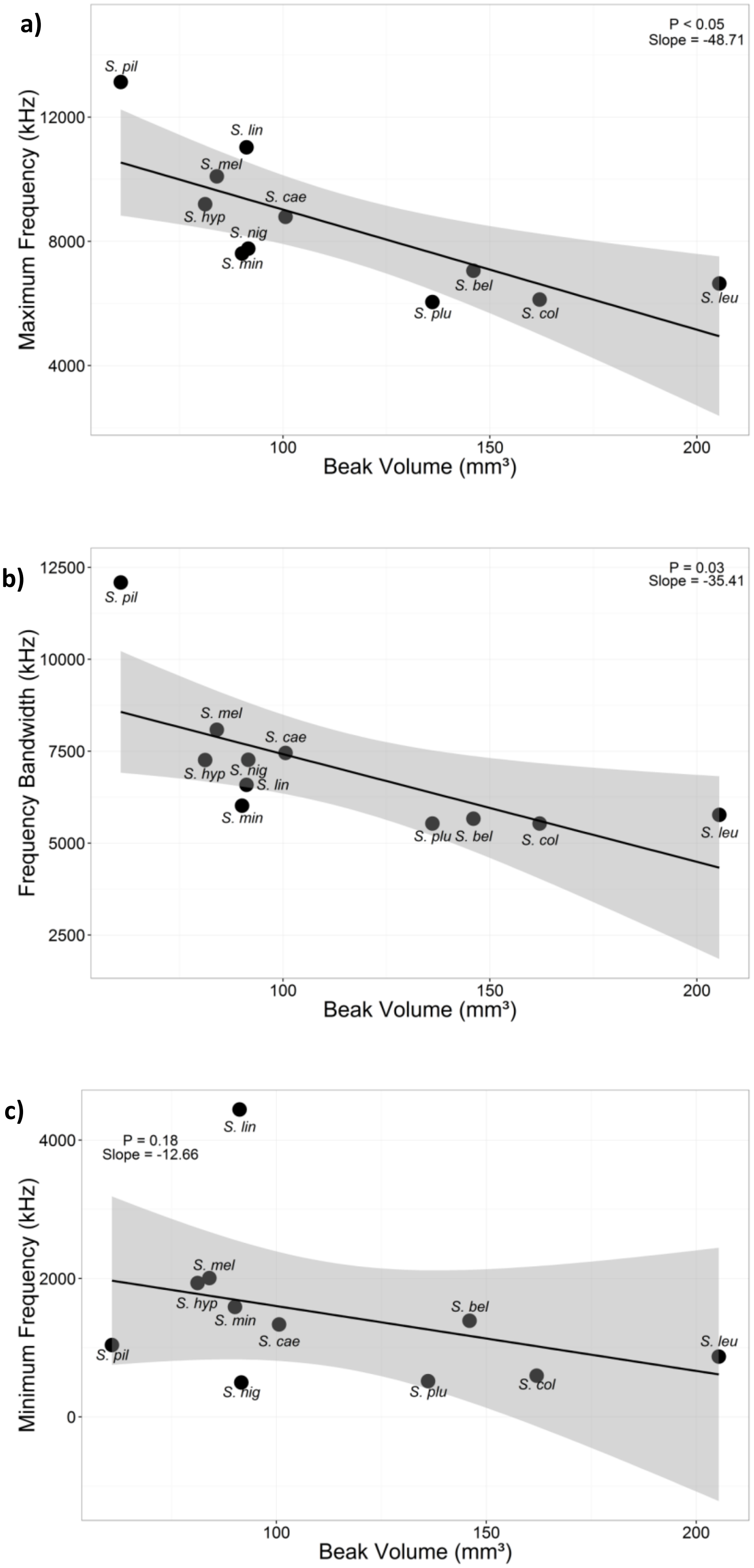

Figure 2 Relation between song parameters and beak volume of Neotropical Seedeaters: maximum frequency (a), frequency bandwidth (b) and a tendency of negative relation for minimum frequency (c).

Evaluations of body mass and beak volume showed a statistically significant association ($P < 0.05$, *Slope* = 21.58) with a positive relation between them (Fig. 3). Concerning to beak volume, *S. leucoptera* had a larger beak and presented greater difference than other seedeaters, on the other hand species with an intermediate beak volume had lower differences (Fig. 4). Beak volume had a widely variation, which *S. pileata* shows the smaller beak (60.8 mm^3), followed by *S. hypoxantha* (81.2 mm^3), *S. melanogaster* (84 mm^3), *S. minuta* (90.1 mm^3), *S. lineola* (91.2 mm^3), *S. nigricollis* (91.6 mm^3), *S. caerulescens* (100.6 mm^3), *S. plumbea* (136.1 mm^3), *S. beltoni* (146 mm^3), *S. collaris* (162 mm^3) and the bigger one, *S. leucoptera* (205.4 mm^3).

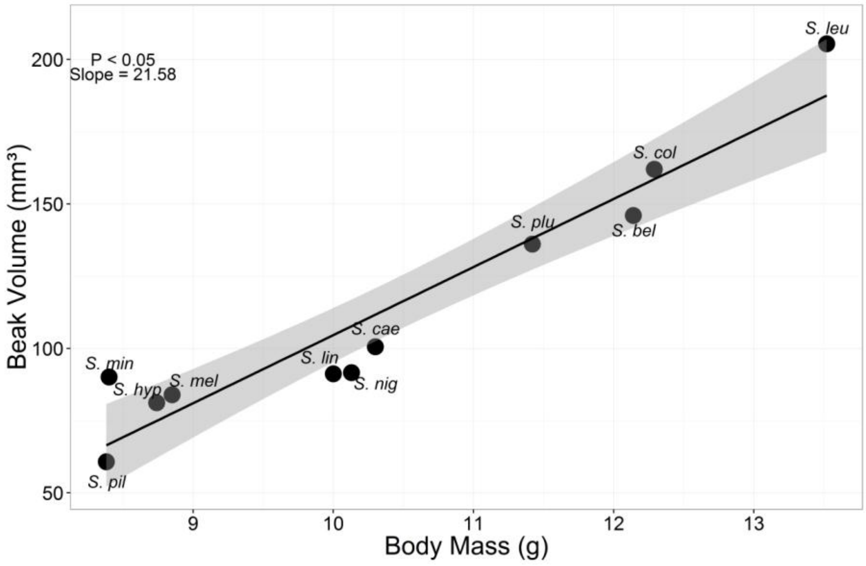

Figure 3 Relation between body mass and beak volume for species of Seedeaters.

We found that beak volume and note rate presented phylogenetic signal ($\lambda \geq 1$), in which closely related species have these traits more similar than the others species studied. As we can see on the Figure 5, closely related species present similar note rate. Minimum frequency presented a statistically significant result either, that λ was very close to one (0.945), demonstrating that this parameter is also similar on closely related species. The others parameters evaluated (peak frequency, high frequency, frequency bandwidth, song duration and number of notes) were not explained by phylogeny.

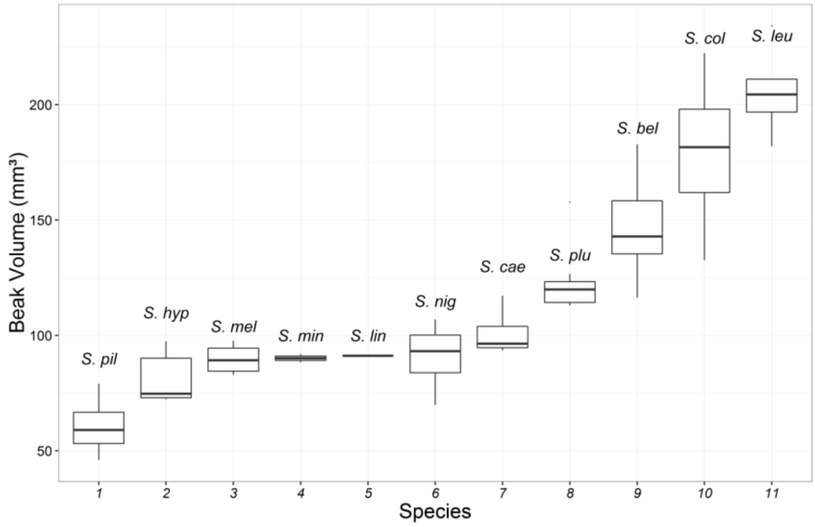

Figure 4 Beak volume avarage in eleven species of *Sporophila*. *Sporophila pileata* (*S. pil*), *S. hypoxantha* (*S. hyp*), *S. melanogaster* (*S. mel*), *S. minuta* (*S. min*), *S. nigricollis* (*S. nig*), *S. caerulescens* (*S. cae*), *S. plumbea* (*S. plu*), *S. beltoni* (*S. bel*), *S. collaris* (*S. col*) and *S. leucoptera* (*S. leu*). The great range of beak volume in *S. col* (*Sporophila collaris*) is related for three different subspecies recognized.

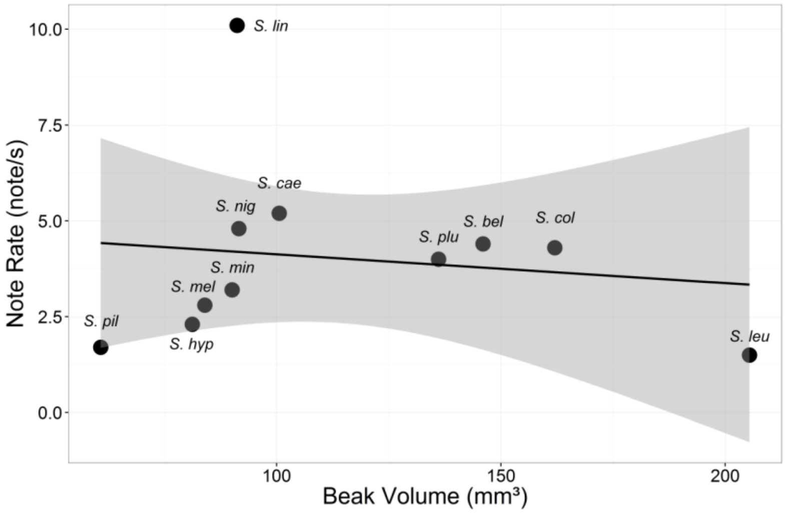

Figure 5 Relation of note rate and beak volume in species of *Sporophila*. It is possible to see the influence of phylogeny on data distribution, in which closely related species presents similar note rate.

DISCUSSION

Many studies have shown that exist constraints on birdsong production, primarily caused by beak features (Podos 2001; Podos and Nowicki 2004; Ballentine 2006; Martin et al. 2011; Derryberry et al. 2012). Our results corroborate this idea, since three variables demonstrates to be influenced by beak volume. Frequency bandwidth and maximum frequency have a negative relation with beak volume, although that, minimum frequency show a tendency for this pattern suggesting that beak volume act as magic trait liming higher frequencies and wider bandwidths. As seen in other groups, birds with larger beaks presented narrow frequency bandwidth and lower frequencies, unlike individuals with small beaks.

In despite of variables by frequency other features have presented limitations over beak volume variation and other parameters did not present this constraint, as note rate. This information demonstrates that *Sporophila* species have a great vocal plasticity to produce a high note rates with large beak, unlike other Oscine birds. Such aspect can be exemplified by the *capuchinos* group, which had smaller beaks and presented a lower note rate than congeners of larger beaks as *S. plumbea*, *S. beltoni* and *S. collaris*. When we verify the phylogenetic signal of song and morphologic parameters we found that beak volume, minimum frequency and note rate are influenced by phylogenetic relationships, which means that closely related species may have similar features of these variables. Referring to note rate this prediction is notable, the groups with greater phylogenetic proximity exhibit similar features, such as the *capuchinos* (*S. pileata*, *S. hypoxantha*, *S. melanogaster* and *S. minuta*) that are clustered, *S. nigricollis* and *S. caerulescens* which have similar note rate to *S. plumbea*, *S. beltoni* and *S. collaris* species closely related to them. *Sporophila lineola*, which is the more external species of our analysis, appear isolated from other species, with a singular trait. *Sporophila leucoptera*, despite of its greatest beak volume showed note rate similar to the group of capuchinos, which it is approaching phylogeneticaly.

Beak volume presented an increase gradiant from *S. pileata* to *S. leucoptera*, within more close phylogenetic species with the exception of *S. leucoptera* that present a larger beak in it group. *Sporophila leucoptera* apears close to *capuchinos* in our tree, however, in a total phylogenetic tree of *Sporophila* it is on a singular branch, with a sister group including *S. peruviana, S. simplex* and *S. telasco,* a group that present larger beaks different from their sister group, what can explain *S. leucoptera* beak volume morphology (Burns et al. 2014).

Different from the well known history of Darwin's finches, Neotropical Seedeaters are a few known large group in which many species coexist in a same area, what can include a sympatric model of speciation. In this scenario where species lives at the same environment and share resources an evolution process may be slowlyer (when compared to allopatric speciation) with a little variation among species, besides being able to exists a low rate of phylogenetic differentiation (Campagna et al. 2010, Paradis 2014). The extreme diversity in Sporophilinae and it evolutional history may be factors that explain some patterns of vocal structure, as we saw for some parameters. *Sporophila* may have a great plasticity in the beak movements, since many species feed on similar resources but present differences in beak size and form besides a widely variation in song structure among them (Rising et al. 2011).

Our study is a first approach in the knowledge of motor constrains of song, in a large group of Oscine birds and with a phylogenetic analysis, and suggests that is still necessary to investigate the divergence of morphological characters that influence birdsong, acting as magic traits, but is also important understand and evaluate the phylogenetic history of the groups to know how they evolve and about which circumstances they pass through diversification. We belive that larger groups, with a widely variation in song structure and beak size are a great model to verify the existence of motor constraint and phylogenetical signal. Evaluate the phylogenetic interference on song structure is essential to understand this constraints or modulators, due to their similarity can be a result of their kindship and no for morphological features that act as magic trait.

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the morphological and song analysis. NSP wrote the article with the contribution from the other authors. There is no conflits or interest from authors to declare.

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CAPÍTULO II

Revista de submissão: Animal Behavior – Conceito CAPES: A1

Does beak volume and bite force influence song structure of sympatric species of Neotropical Seedeaters (Thraupidae: *Sporophila*)?

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Song structure can be constrained by morphological characters, such as beak and body size. Many studies between species have shown that birds with larger beaks and body size produce lower frequencies songs and narrow frequency range with low note rate, unlike smaller ones. At south Brazil *Sporophila beltoni*, *Sporophila caerulescens* and *Sporophila hypoxantha* occur in sympatry and have a difference among beak size as putative segregation of based-seeds diet. Is predicted that these birds segregate acoustic space to reduce the overlap of their songs, what is a possible result of the strength of natural selection. In this study, we have tested if bite force and beak volume constraint song structure of these three species and evaluated what differences they have in song characters that may differ in acoustic space. Songs of five adult males of each species were recorded in their breading season and their beaks and bite forces were measured. For song structure were evaluated traditional physic song parameters. The use of acoustical space seems to be singular in each species for note rate and song duration, features that differ among them. We found that *S. beltoni* and *S. hypoxantha* have more differences between them in all the analyzed parameters but for the relationship between beak volume and song structure we observed that *S. caerulescens* had a positive relationship with this variable and three song parameters: maximum/minimum frequencies and frequency bandwidth. Maximum frequency also had a positive relation with bite force in *S. beltoni* and *S. caerulescens*. For bite force, the other parameters evaluated shown conservative results. Our results suggest that for these three sympatric species beak volume and bite force do not limit frequencies (maximum and minimum) production and note rate. These birds demonstrate that they can modulate their songs with a widely size and strong of beak, different from other cone beak finches.

Keywords

Birdsong, Oscines, vocal performance, acoustic communication, ecological segregation, animal behavior.

Acoustic signals are extremely important for specfic recognition, acting as a reproductive barrier in the speciation process (Marler, Slabbekoorn & Bowman, 2004; Catchpole & Slater, 2008). In Oscine birds is through song that they attract mates, alarm others from predators and defend their territories. Oscines songs are learned as a complex behavior that is subject to multiple pressures such sexual selection, natural selection and rapid diversification/evolution (Kroodsma, 2003; Catchpole & Slater, 2008; Wilson, Bitton, Podos & Mennill, 2014).

The sound produced by birds is generated in a specialized organ, the syrinx, however, the final structure of this acoustic signal is also influenced by others characters (Catchpole & Slater 2008). Larynx, trachea and beak are anterior structures of vocal tract that act as an acoustic resonance filter for sound and can shape it as well (Nowicki, 1987; Westneat, Long, Hoese & Nowicki, 1993; Podos, 2001; Podos, Huber & Taft, 2004; Podos & Nowicki, 2004). Studies have shown that birds with smaller body size have been increased frequencies produced and the opposed happened to bigger birds (Ryan & Brenowitz, 1985; Podos & Nowicki, 2004; Doucet, Knox & Mennill, 2011; Giraudeu, et al., 2014).

Bite force and beak size can be associated with birds capacity to move the jaws while they are singing, constraining consequently the song produced (Ratcliffe & Grant, 1985; Hoese, Podos, Boetticher, Nowicki, 2000; Grenier & Greenberg, 2005; Christensen, Kleindorfer, & Robertson, 2006; Huber & Podos, 2006; Derryberry, et al., 2012). Beak morphology is generally a byproduct of the adaptation of feeding habit, related to environments, food availability and inter-specific competition. For example, birds that feed hard and big seeds have stronger and larger beaks than those that feed on insects (Herrel, Podos, 2001; Podos, Huber & Henryl, 2005; Genbrugge, et al., 2011). This characteristic influence on how widely and rapidly the bird can move its beak (Herrel, Podos, Vanhooydonck & Hendry, 2009; Genbrugge, et al., 2011). The same pattern observed in the relationship between body size and frequencies is also seen between beak size and bite force.

Birds with larger beaks and stronger bites may produce songs with lower frequencies, narrower frequency bandwidth and less note rate (Westneat, et al., 1993; Podos & Nowicki, 2004; Catchpole & Slater, 2008; Giraudeu, et al., 2014; Hoese, et al., 2000; Mason & Burns, 2015). Beak gape is associated with song structure variation; an open beak modifies the resonance of vocal tract providing higher frequencies and frequency bandwidth produced as opposite to closed beak that provide lower ones (Ballentine, 2006). The movement to open and close beak is a motor constraint of producing repetitions in songs, which birds that can open and close the beak faster generally produces higher note rates (Podos, et al., 2004). There is a trade-off between rate note and frequency bandwidth, this relationship presents a triangular distribution in some species which only songs with narrow frequency bandwidth can produce higher note rates, while narrow and higher frequencies bandwidth produce a lower note rates (Podos, 1997; Podos, 2001, Wilson, et al., 2014).

When a bird sings, it wants to be heard by a receiver that may be a female, it group, a predator or other males. In order to this sound reach them, there are many obstacles that can interfere this transmission, as noise, the acoustic proprieties of their environments and other animal songs (Morton, 1975; Linskens, et al., 1976; Wiley & Richards, 1978; Richards & Wiley, 1980; Endler, 1992). Species that coexist may adapt their songs to be heard and avoid an overlap of acoustic space. There is some alternatives to overcome this overlap, one way is to increase maximum frequency of the song, when the noise is characterized by low frequencies, or an alternatively is to invest in lower frequencies which have longer wavelengths and diffract the obstacles more easily than higher frequencies (Wiley & Richards, 1978). Another option that sympatric species have to modulate the sound is to increase trill rate, producing longer notes and inter-notes intervals or sing at higher amplitudes (Morton, 1975; Brumm & Zollinger, 2011).Understanding the characters that limit vocal signals is thus an important aspect to clarify diversification and evolution of species.

The *Sporophila* (seedeaters) belongs to Thraupidae (Passeriformes) and includes about 34 species from Subtropical and Tropical America that have clades with recent radiation (Burns, et al., 2014; Barker, Burns, Klicka, Lanyon & Lovette, 2015). These group males have different plumage between species and most females are similar, presenting olive or brown color (Meyer de Schauensee, 1952; Mason & Burns, 2013). This genus exhibit a great diversity in beak size, from small and slight (e.g., *Sporophila pileata*) to big and strong beaks (e.g., *Sporophila leucoptera*). Furthermore, some seedeaters have the rate of diversification comparable to that of Darwin's finches, which makes this a potentially useful study system of divergence and evolution in mainland (Burns, et al., 2014). As well as Darwin's finches, the seedeaters are a monophyletic group (Mason & Burns, 2013; Burns, et al., 2014). However, this genus passed through the continental speciation process, with coexistence of species that lives currently in sympatry. In a sympatric speciation model species are adapted to different sets of ecological traits, thereby is expected that species that coexist uses different acoustic spaces (Grant & Grant, 1979).

Many studies have focused their researches in the link between beaks size and shape with song (Nowicki, 1987; Westneat, et al., 1993; Podos, 2001; Hoese, et al., 2000; Podos & Nowicki, 2004). However, most of them have worked with a particular group, Darwin's finches, which have gone through a very specific process (Lack, 1947; Bowman, 1979; Podos, 2001; Podos & Nowicki, 2004; Podos, et al., 2004; Huber & Podos, 2006). Morphological adaptation of beak is often followed by song divergence, but does it happens in sympatric birds of the same genus with a widely variation in song structure? Do they adjust acustic parameters to be heard due to the presence of related species that overlap birdsongs? The objective of this study was to test if the song structure is constraint by beak volume and bite force among three sympatric species of *Sporophila*. Besides that we compared song structure of these three species to verify how they differ in the acoustic space, since they coexist in nature.

METHODS

Study Species and Sites

We work with three sympatric species that breeds in the same habitat, *Sporophila beltoni*, Tropeiro Seedeater; *Sporophila caerulescens*, Double-collared Seedeater and *Sporophila hypoxantha*, Tawny-bellied Seedeater. The first species was described recently, as a split of *Sporophila plumbea*. It breeds from northern of Rio Grande do Sul to the northeast of Paraná and it has a fragmented distribution, being considered endangered status of conservation due the decline of its population caused by habitat loss and bird trappers (Repenning & Fontana, 2013). It is the bigger species of our study, being up to 12 g body mass and 119 mm total length (Repenning & Fontana, 2013). *Sporophila caerulescens* has a widely distribution, a partial migrant, which breeds in Argentina, Paraguay, Uruguay and parts of Brazil. It has a least concerned status by IUCN (2016) and is the medium species of this work, measuring on average 115 mm and 10.5 g of body mass. The *S. hypoxantha* belongs to a group of species that has a rapid and recent radiation, the capuchinos (Campagna, Silveira, Tubaro & Louugheed, 2013). There are five allopatric population recognized ad we limit the study on easternmost population that breeds in highlands of south Brazil (Areta & Repenning, 2011). It’s the smaller bird among the three studied, measuring 8.5 mm and 8.8 g of body mass (Franz & Fontana, 2013). We did our field work at the municipalities of Bom Jesus (28°40′S, 50°28′W) and boundaries of Jaquirana (28°52′S, 50°27′W), state of Rio Grande do Sul, Brazil, where the three species breed in sintopy. This region is known as upland grasslands that belong to Atlantic Forest Biome in southern Brazil.

Field Work

We recorded five adult male territorial songs for each species during five days in December 2015, in their breeding season. We selected adult territorial males singing advertisement songs. Records of songs were made with a standard distance from the individuals (~20 m) using a Teac Tascam digital recorder Professional DR 680, with external microphone Sennheiser ME62 associated with a Telinga parabola (21.5in/6.1in). All sounds recorded for this study will be archived at the Macaulay

Library of Natural Sounds, Cornell Lab of Ornithology (Ithaca, NY). After that, males were captured with a mist net using the recurse of plauback for take morphometric measurements and to test bite force. We banded all birds with metallic and color rings to individualize them. We measured three parts of the beak (height, width, and length), total length and body mass with a metallic caliper rule (Mitutoyo) and a precision scale 0.01 mm and dynamometer Pesola Swiss Micro online 20060 precision 0.5 g (Ballentine, 2006; Roos, 2010). The beak volume was calculated as a cone volume [$v= (\pi.r^2.h)/3$], where we used r = beak height/2 and h = beak length (exposed culmen). For measure the bite force we used a pressure sensor Flexiforce – Tekscan (Freeman & Lemen, 2008) linked to a multimeter (2V) mounted in a costume-built holder. It was calibrated with known masses (g) that we could transformed the values obtained from volts to grams. Such equipment was developed together with the Physic department of our University (PUCRS), so that it was of low cost, easy handling and transporting during the field work. For test the bite force each bird was stimulated to bite ten times the platform by a pressure sensor (Fig.2), after that birds were released.

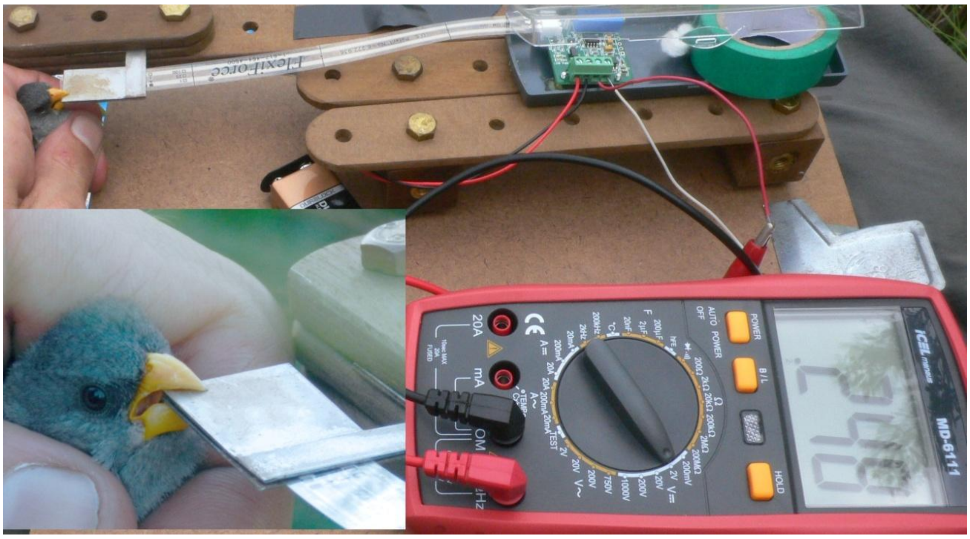

Figure 1 Bite force experiment using a pressure sensor linked to a multimeter. In the detail a specimen of *S. beltoni* bitting the equipament.

Sound Processing

All acoustic measurements were made with Raven Pro 1.5 using the following spectrogram parameters [Window-Type: Hann; Size: 512 Samples (10.7 ms); Time grid-Overlap: 75%; Frequency grid-DFT size: 1024 samples and Bandwidth 270 Hz]. For each bird we analyzed three songs that we calculated mean values for any parameters. We evaluated eight acoustic variables: song duration, number of notes, note rate (note/s: number of notes per second), frequency bandwidth, peak frequency, maximum and minimum frequencies and the percentage of different notes in a song, as the difference between singular notes and total notes. We used the amplitude spectra to calculate the frequency bandwidth as the difference between maximum and minimum frequency, using a -24 dB relative to the peak amplitude frequency in song (Podos, 1997; Podos, 2001). Variables by time (number of notes, different notes, time and note rate) were measured with an oscillogram, which we delineate each song and select with an on-screen cursor for measure it duration. We classify the syllables/notes based on visual inspection in to the discrete categories.

Statistical Analysis

We estimated performance constraint using simple linear regression for each data set and each species, which aims to describe how song structure is affected by beak volume and bite force. Furthermore we evaluate how beak volume and bite force are related, using the same analysis but using allometric values for this search (West, Brown & Enquist, 1997). For bite force values we selected the five strongest bites and averaged them to estimate the pattern of bite force per individual (Herrel, et al., 2005). To evaluate the use of song space by the species, we compared song duration, frequency bandwidth, number of notes, note rate, maximum and minimum frequencies using univariate analysis. We used the Shapiro test to check the normal distribution of data, followed by ANOVA ($P < 0.05$) and *post hoc* Tukey test. For non-normal distribution we process data using Kruskal-Wallis and Dunn test as *post hoc*. All statistical analyzes were performed using R (R Core Team 2016). We used package 'arm' for simple linear regression; 'car' for ANOVA; 'coin' and 'dunn.test' for Kruskal-Wallis and 'ggplot2' for graphs.

Ethical Note

All of the bird manipulations were realized under Brazilian's law. We had all licenses from National Center for Research on the Conservation of Wild Birds (CEMAVE, nº 361788), Ethical Council of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) and permit from ICMBio by Brazilian System and Information on Biodiversity (SISBIO, nº 36881).

RESULTS

Interespecific Variation

Our study had a small, medium and large species model, in which *S. beltoni* had the greater beak volume and bite force, followed by *S. caerulescens* and *S. hypoxantha*, the same pattern is observed on their body mass (Tab. 1). Five of our song and morphological variables presented statistically significant differences between at least two species. *Sporophila beltoni* and *S. hypoxantha* showed more differences between them, beak volume (Kruskal-Wallis, $P = 0.008$), bite force (ANOVA, $F2,8 = 9.68$, $P = 0.003$), note rate (ANOVA, $F2,7 = 54.64$, $P < 0.001$) and song duration (Kruskal-Wallis, $P = 0.027$). *Sporophila hypoxantha* and *S. caerulescens* had three statistically significant results: note rate (ANOVA, $F2,7 = 54.64$, $P < 0.001$), song duration (Kruskal-Wallis, $P = 0.001$) and percentage of different notes (Kruskal-Wallis, $P = 0.008$); *Sporophila caerulescens* and *S. beltoni* also presented two statistically significant differences between them: bite force (ANOVA, $F2,8 = 9.68$, $P < 0.014$) and beak volume (Kruskal-Wallis, *P* = 0.036). The frequency parameters (peak, minimum/ maximum frequencies and frequency bandwidth) and number of notes did not present statistically significant differences (*P* > 0.05) between species, as we can see in table 2. Beak volume, bite force, note rate, song duration and percentage of different notes were the most striking differences between the three species (Fig. 2).

Table 1 Overview of morphological data for the three species analyzed in this study (mean±SD) .

Species	n	Beak volume mm³	Max. bite force (g)	Body mass (g)
S. beltoni	5	176.5±23.2	63.5±34.9	12.2±1.1
S. caerulescens	5	98.5±10.6	31.0±46.5	10.1±1.1
S.hypoxantha	5	78.8±35.9	23.5±31	8.6±0.8

Table 2 ANOVA and Kruskal-Wallis results for all parameters that did not showed significant differences between *S. beltoni* (*S. bel*), *S. caerulescens* (*S. cae*) and *S. hypoxantha* (*S. hyp*).

			P- value		
			S. cae – S. bel	*S. hyp – S. bel*	*S. hyp – S.cae*
ANOVA	Peak Frequency	F*2,7* = 0.15	0.977	0.953	0.873
	Number of Notes	F*2,7* = 23.72	0.977	0.953	0.873
	Frequency Bandwidth	F*2,8* = 0.62	0.704	0.627	0.991
Kruskal-Wallis	Mininum Frequency		0.268	0.31	0.235
	Maximum Frequency		0.857	0.471	0.465

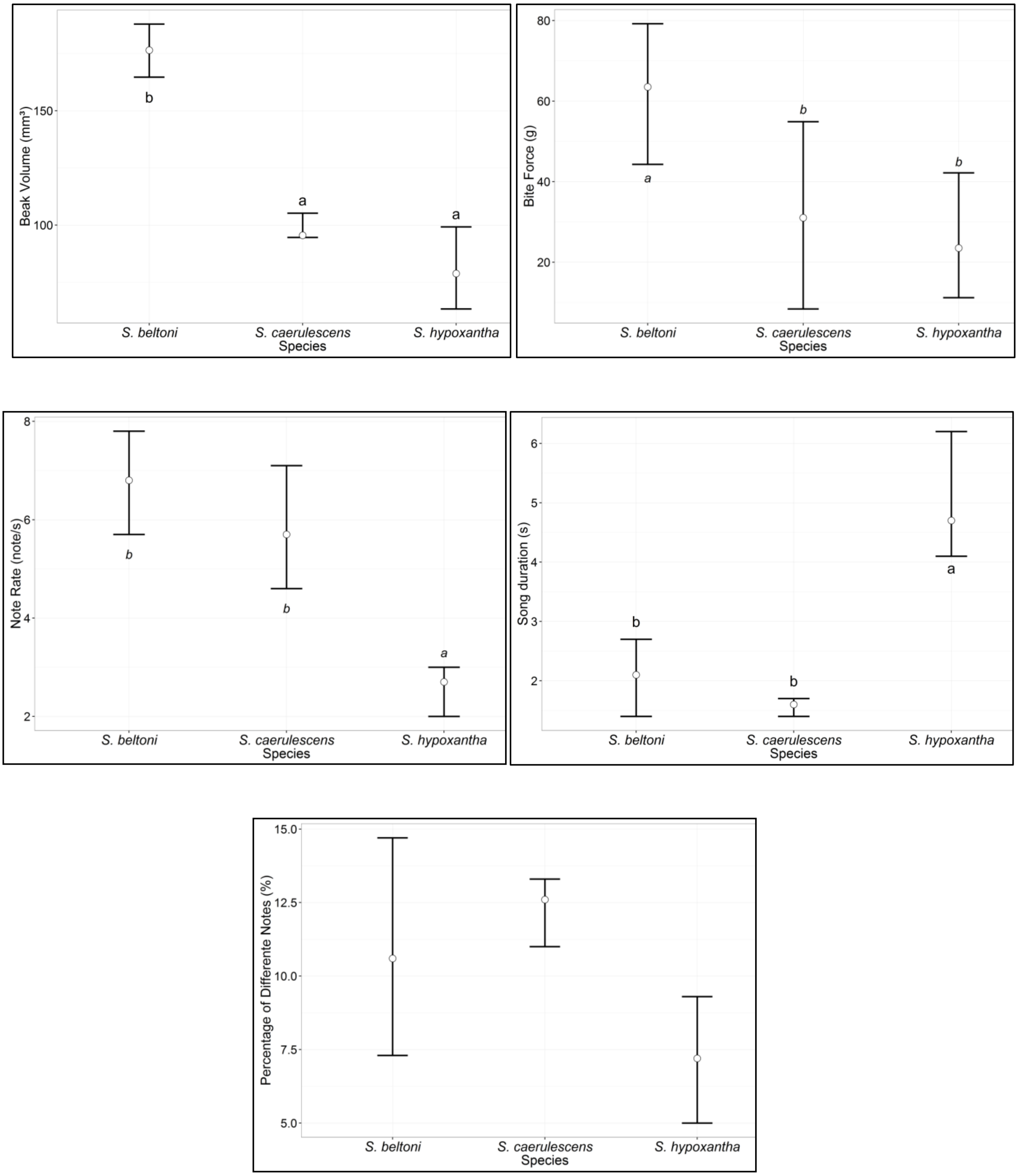

Figure 2 Intraespecific variation of beak volume, bite force, note rate, song duration and percentage of different notes between three simpatric species of Seedeaters with the differences between them. Refer to the percentage of different notes in the song, only *S. hypoxantha* and *S. caerulescens* presented significant difference ($P < 0.05$).

Intraespecific Variation

In the relationship between beak volume and song structure (Simple Linear Regression) *S.caerulescens* was the only species that showed statistically significant results, maximum frequency (P = 0.03, $R2$ = 0.82, $F1,3$ = 13.49) and frequency bandwidth (P = 0.03, $R2$ = 0.81, $F1,3$ = 13.63) were strongly explained by the morphologic data, that birds with larger beaks exhibited higher frequencies and larger frequencies bandwidth. *Sporophila beltoni* and *S. hypoxantha* do not exhibited significant results ($P > 0.05$) for beak volume and song parameters (Tab. 2). Referring to the analysis of bite force, none of the variables were explained by this morphological data (Tab. 2). Despite this, maximum frequency showed a tendency of positive relationship with bite force in *S. beltoni* (P = 0.08, $R2$ = 0.70, $F1,3$ = 6.874) and *S. caerulescens* (P = 0.18, $R2$ = 0.51, $F1,3$ = 3.093). We tested the association between note rate and frequency bandwidth in which none of the three species presented relationship between these two parameters. We additionally tested how bite force is explained by beak volume and we found that they had a statistically significant result (P = 0.002, $R2$ = 0.51, $F1,3$ = 13.72) with a positive relationship, which birds with larger beaks presented strongest bite force (Fig. 5).

Table 2 Simple linear regressions of beak volume and bite force as dependent variables comparing to parameters of song structure, statistically significant results are in bold.

	S. beltoni			*S. caerulescens*			*S.hypoxantha*		
Beak Volume	*P* - value	*R2*	*Slope*	*P* - value	*R2*	*Slope*	*P* - value	*R2*	*Slope*
Peak Frequency	0.25	0.40	0.31	0.25	0.40	0.03	0.79	0.03	-0.80
Minimum Frequency	0.31	0.33	9.53	0.13	0.58	16.19	0.54	0.14	3.18
Maximum Frequency	0.29	0.36	72.56	**0.03**	**0.82**	**552.30**	0.57	0.13	-54.81
Number of Notes	0.47	0.19	0.19	0.99	<0.01	-0.00	0.43	0.21	0.03

Note Rate	0.79	0.05	0.01	0.80	0.03	-0.03	0.64	0.08	-0.00
Frequency Bandwidth	0.99	<0.01	1.78	**0.03**	**0.81**	**536.10**	0.55	0.13	-57.99

Bite Force	*P* - value	*R2*	*Slope*	*P* - value	*R2*	*Slope*	*P* - value	*R2*	*Slope*
Peak Frequency	0.35	0.29	-0.01	0.33	0.31	-0.17	0.67	0.07	0.05
Minimum Frequency	0.33	0.62	8.70	0.24	0.42	3.51	0.24	0.42	-5.41
Maximum Frequency	0.08	0.70	67.93	0.18	0.51	110.68	0.60	0.10	52.67
Number of Notes	0.37	0.27	-0.15	0.94	<0.01	0.00	0.57	0.12	0.02
Note Rate	0.18	0.50	-0.04	0.84	0.01	0.00	0.38	0.26	0.01
Frequency Bandwidth	0.22	0.44	73.04	0.18	0.51	107.16	0.58	0.13	58.09

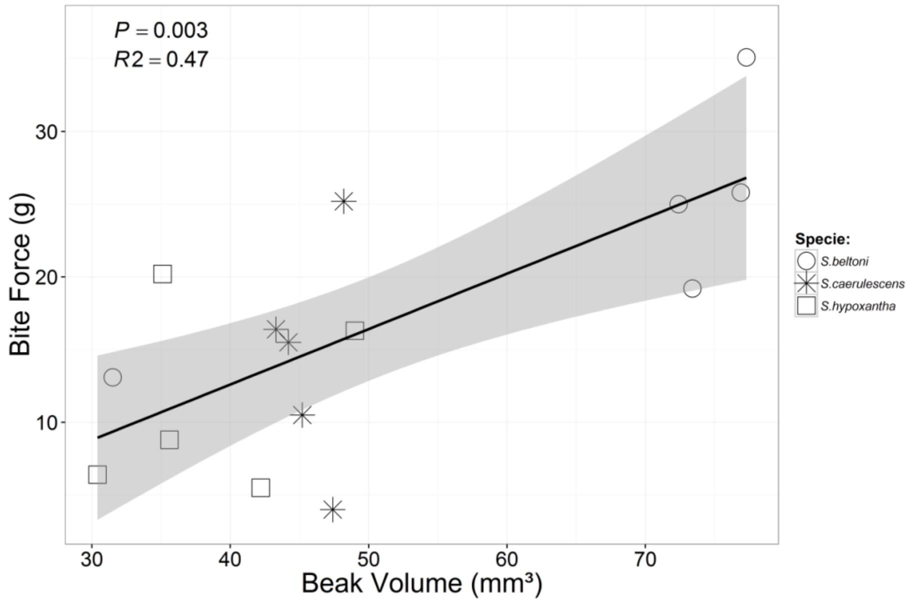

Figure 5 Relationship between the beak volume and bite force in *S. beltoni*, *S. caerulescens* and *S. hypoxantha*.

DISCUSSION

Beak size and bite force are known as a motor constraint for vocal performance in some Oscine and Suboscine birds (Podos, 1997; Podos, 2001; Podos & Nowicki, 2004; Ballentine, 2006; Herrel, et al., 2009; Derryberry, 2012; Giraudeu, et al., 2015).

In our test of this prediction *S. beltoni* showed statistically significant difference in beak size and bite force compared with the smaller species; however, the variation in song parameters was slight. There were no statistically significant differences between song frequencies of the syntopic individuals of three species, nonetheless, note rate and song duration presented divergences around them what can be a way that they modulate sound and avoid an overlap of their song due the competition for acoustic space (Morton, 1975; Brumm & Zollinger, 2011).

Among the acoustic parameters analyzed, two of them were particularly different between the three species, factors that can help them on the competition for acoustic space, since they interbreed in the same habiat and have closely territorials. Note rate and song duration were significantly different between *S. hypoxantha* and the other two seedeaters. The smallest one, *S. hypoxantha* had a longer song with a lower note rate unlike *S. beltoni* that presented higher note rate in a shorter time, in despite of *S. caerulescens* does not showed significant difference with *S. beltoni*, its song is shorter and had a lower note rate that the other one. This pattern is the opposite that observed in other groups, such as Darwin’s finches, sparrows and woodcrepers (Podos, 2001; Podos & Nowicki, 2004; Ballentine, 2006; Derryberry, 2012). In addition to that, phylogenetic relationships may explain some song parameters, as note rate in seedeaters (Porzio, Repenning & Fontana, in prep.) For many taxa there is a divergence in variables of frequency (maximum/minimum and frequency bandwidth) according to the variation of beak size, feature that did not appear in our species of study.Although these three seedeaters do not show great segregation for these parameters, in a bigger scale of this group, with a widely variation in beak shape and size we can observe a relation between beak volume and song structure (Porzio, Repenning & Fontana, in prep).

The model observed mainly in Darwin’s finches predicts that birds with larger beaks and stronger bite forces may present constraints in note rate, maximum and minimum frequencies and frequency bandwidth (Podos, 1997; Hoese et al., 2000; Podos, 2001; Podos et al., 2004). In our study *S. hypoxantha* presented the structure of song more stable than the others, therefore did not demonstrated relation with beak volume or bite force. Besides that, this species also presented a constant song, with a higher pattern in note rate and song duration. This pattern of stereotyped song was already observed in a study with *S. hypoxantha* populations and it is more common in capuchinos (Repenning, Rovedder & Fontana, 2010; Areta & Repenning, 2011).

Althoght of *S. beltoni* did not present statistically significan results between beak and song, we observed that minimum and maximum frequencies tend to a positive relation going against the classic literature data (Podos, 2001; Podos & Nowicki, 2004; Ballentine, 2006; Derryberry, 2012). On the other hand note rate analysis showed a predicted negative relation between variables suggesting that this character is constricted by bite force for this species. However, for seedeaters note rate present a phylogenetic signal and do not have a statistically significant result when related to beak volume, as observed in our study (Porzio, Repenning, Fontana in prep.). *Sporophila beltoni* have the capacity to mimic besides learning songs and for that, it presents a great vocal plasticity (Repenning & Fontana, 2013). This species showed a widely variation in number of notes, song duration and consequently note rate, demonstrating no limitation to produce it or to produce variation in frequencies, similar to the smaller species that shows no constraints.

The medium size species studied, *S. caerulescens* was the species that has more statistically significant results that are relevant concerning the relation between beak and song structure. Unlike the other species evaluated here, just a few studies are known about the song of *S. caerulescens*, although it is the most common among these three. In both analysis between bite force and beak volume related to song structure there were relevant results for minimum/maximum frequencies and frequency bandwidth. We found a positive relation between morphologic and song data as opposite to other groups already studied, showing that beak volume or bite force may not be a constraint for these three seedeaters. However, an analysis with a large group of seedeaters species demonstrates that maximum frequency and frequency bandwidth were limited by beak volume, in which birds with larger beaks produced lower maximum frequency and narrow frequency bandwidth (Porzio, Repenning & Fontana, in prep.). Our study suggests that the songs of these three sympatric species are not constraint by bite force or beak volume, so they can modulate their frequencies similarly between them and note rate is not affected by beak characters either.

Many studies have shown that larger beaks with stronger bites are physically incompatible to make quick opening and closing movements, affecting the note rate and frequencies produced (Hoese, et al., 2000; Podos & Nowicki, 2004). We showed a different pattern for these relationships. About sources competition, our results shows that these three species have different song structures that may be a facilitator for their coexistence to avoid song overlapping. However, is already known that main different feature (note rate) in this case, is due to their phylogenetic relationships (Pozio, Repenning & Fontana, in prep.). Further studies with species closely related that present a wide variation in beak size and shape would help to verify the influence of morphology on song structure. It is also important to search about birdsong evolution, since phylogeny can explain many of the song parameters among the species that morphologic characters cannot.

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Conclusões gerais

A principal ideia do nosso trabalho foi testar a predição de que o bico atua como um limitador na estrutura do canto das aves. Este padrão vem sendo observado e estudado especialmente em um grupo particular, os tentilhões de Darwin. Estas aves apresentam uma ampla variação no tamanho e formato do bico o que faz delas um ótimo grupo para testar essa relação. No entanto, elas passaram por um processo singular de especiação em ilhas além de ser um grupo pequeno, incluindo cerca de oito espécies.

Sporophilinae foi um excelente táxon para testar a relação entre bico e parâmetros do som, já que conta com muitos representantes, contendo aproximadamente 34 espécies com uma ampla variação no tamanho do bico, além de ser um grupo monofilético que apresenta uma divergência recente no clado dos caboclinhos, o que torna o estudo ainda mais interessante (Rising et al. 2011; Burns et al. 2014). No capítulo I, analisamos um grupo maior de *Sporophila* e observamos que assim como os tentilhões de Darwin, o bico dos papa-capins atua como um limitador na largura de banda e frequências máximas/mínimas dos indivíduos e que estas características coevoluíram, no qual aves com bicos maiores produzem frequências máximas inferiores comparado às aves com bicos menores. No entanto alguns parâmetros não apresentaram limitações com o volume do bico (ex. taxa de notas). Esta informação nos mostra que nem todos os parâmetros do som são limitados por este caractere morfológico, sugerindo que ainda tem muito para se descobrir a respeito dos limitadores e moduladores do som em aves. Além disso, encontramos que o volume do bico, a taxa de notas e a frequência mínima são parâmetros que apresentam sinal filogenético, o que demonstra que existem mais fatores atuando sobre a estrutura do canto neste grupo.

No segundo capítulo testamos a relação de dois caracteres morfológicos, pressão da mordida e volume do bico sobre a estrutura do canto, e avaliamos a estrutura do canto de três espécies simpátricas para entender como elas organizam seus cantos de maneira que não haja sobreposição acústica, já que elas coexistem. Diferente do nosso primeiro estudo, para estas três espécies (*S. beltoni*, *S. caerulescens* e *S. hypoxantha*) não houve padrão de limitação do canto. Tivemos uma surpresa em *Sporophila caerulescens*, esta foi a espécie que apresentou mais resultados significativos, no entanto a relação entre os caracteres morfológicos e acústicos foi oposta aos que outros trabalhos haviam encontrado. Largura de banda e frequências máxima e mínima apresentaram uma relação positiva com a pressão da mordida e o volume do bico, no qual indivíduos com bicos menores produziram cantos com frequências máximas inferiores e largura de banda mais restrita comparado aos indivíduos de bicos maiores. Este resultado indica que caracteres morfológicos como limitadores na estrutura do canto não são um padrão generalizado em *Oscines*, sendo então necessário investigar cada grupo em particular para entender seus mecanismos e a evolução dos mesmos. Sobre a divisão do espaço acústico nós encontramos que a taxa de notas e duração do canto são significativamente diferentes entre *S. hypoxantha* e as outras duas espécies, e apesar de S. *caerulescens* não apresentar diferença significativa com *S. beltoni*, seus cantos têm estruturas diferentes, com maior taxa de notas na segunda espécie. Estas diferenças nos parâmetros do som sugerem como estes papa-capins evitam a sobreposição enquanto estão cantando e competindo pelo espaço acústico. Apesar disso, como nós encontramos no capítulo I, a taxa de notas é uma variável que apresenta sinal filogenético, mostrando que este parâmetro é resultado da história filogenética destas espécies.

Nossos resultados demonstram que ainda tem muito a ser estudado sobre a estrutura do som, especialmente em aves *Oscines*. Existem muitos tipos de cantos e diferentes morfologias do bico além das relações filogenéticas, o que torna necessário verificar a influência de todos estes fatores sobre a estrutura do som. Muitas aves têm ampla plasticidade vocal e muitas delas podem apresentar plasticidade morfológica, na movimentação do bico, por exemplo, de maneira que este caractere não irá limitar o som. Estudos com grupos maiores que verifiquem a influência de caracteres morfológicos e filogenéticos sobre a estrutura do canto irão ajudar no entendimento dos mecanismos “limitadores” e na evolução do canto através dos grupos de aves *Oscines*.