UNIVERSIDADE FEDERAL DO PARANÁ
VIVIANE DESLANDES DO NASCIMENTO

A EVOLUÇÃO DO CANTO EM AVES: INTEGRANDO MORFOLOGIA, FILOGENIA E AMBIENTE.

CURITIBA

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A EVOLUÇÃO DO CANTO EM AVES: INTEGRANDO MORFOLOGIA, FILOGENIA E AMBIENTE

Tese apresentada como requisito
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RESUMO GERAL

A comunicação acústica tem sido um marco na evolução das aves. Estudos sobre evolução do canto geralmente levantam o papel das características morfológicas influenciando o aparato de produção do canto, estrutura do habitat causando barreiras à transmissão do canto, partilha de nicho acústico para evitar sobreposição de sinais entre espécies e deriva cultural durante o processo de aprendizagem. O aumento na disponibilidade de informações sobre a relação filogenética entre espécies, informações ecológicas e a disponibilidade de gravações de alta qualidade permite investigar como estes fatores contribuem para moldar o canto dentro de uma perspectiva macroecológica. Neste estudo foram utilizados métodos filogenéticos comparativos e estatística espacial a fim de testar hipóteses clássicas de limitação (ambiental e morfológica) sobre a estrutura do canto de aves suboscines (famílias Thamnophilidae, Tyrannidae e Pipridae) e oscines (famílias Parulidae e Turdidae). No primeiro capítulo nós testamos a Hipótese de Adaptação Acústica (HAA) (limitação ambiental) em uma ampla escala utilizando uma medida quantitativa de vegetação, a Normalized Difference Vegetation Index (NDVI). Após controlar os efeitos filogenéticos e espaciais sobre os parâmetros do canto, somente Thamnophilidae e Tyrannidae exibiram o padrão esperado segundo a HAA, relacionado à frequência do canto, com uma relação negativa entre frequência e NDVI: cantos em ambiente florestal apresentaram menores valores de freqüência do que cantos em ambientes abertos. A maioria dos modelos com resultados significativos incluiu massa corporal, indicando a influência da morfologia como uma forte limitação sobre a freqüência do canto. Por outro lado, características temporais do canto não foram afetadas pela vegetação. No segundo capítulo nós resumimos a variação interespecífica nas características do canto dessas famílias utilizando análise de componentes principais (PCA) e ajustamos modelos de evolução alternativos (White noise, Brownian motion, Ornstein-Uhlenbeck and Early burst) sobre os scores dos componentes principais do canto. O primeiro PC apontou por aproximadamente 40% da variância em todas as famílias, indicando que o principal eixo de variação da evolução do canto envolve mudanças na freqüência do canto. Mais ainda, os modelos mais simples (White noise e Brownian motion) mostraram melhor ajuste para a maioria dos componentes principais do canto. Finalmente, no terceiro capítulo nós testamos se estas famílias experimentam um balanço entre a variação na freqüência do canto (frequency bandwidth- FB) e a taxa de repetição de elementos (notas) do canto (song rate – SR), já demonstrada em outros taxa. Nós utilizamos dois métodos de regressão para estimar essas relações: *upper bound e quantile regression*. Os resultados da *upper bound regression* foram afetados pelos intervalos estabelecidos em SR. Utilizando intervalo de 1 Hz somente Pipridae exibiu a relação negativa esperada entre FB e SR, mas utilizando intervalo de 2 Hz, Thamnophilidae, Pipridae e Turdidae mostraram resultados significativos. Uma vez que as regressões pelos métodos *upper bound* e *quantile* tiveram resultados conflitantes nós utilizamos análise de regressão filogenética (PGLS) para testar o efeito do tamanho do bico e massa corporal (limitação morfológica) diretamente sobre FB e SR. As medidas do bico foram resumidas utilizando análise de componentes principais e os *scores* dos componentes foram posteriormente utilizados nas análises PGLS. Em quatro famílias todas as medidas do bico foram reduzidas no primeiro componente principal, somente em Pipridae foram necessários dois eixos para representar o tamanho do bico. Os modelos de melhor ajuste apontados pela PGLS foram os que incluíram a interação entre o PC do bico e o log da massa corporal. A morfologia do bico e massa corporal afetaram FB e SR em Thamnophilidae e Parulidae. Em Thamnophilidae bicos pequenos e aves mais leves produzem taxas de repetição mais rápidas e maior variação na faixa de freqüências produzidas, consistente com a hipótese de limitação morfológica sobre a produção do canto. Concluindo, os resultados desta tese provêm evidências para os seguintes princípios gerais a respeito da evolução do canto: (1) as características do canto relacionadas à freqüência são limitadas pelo ambiente e morfologia. Entretanto, para a maioria das famílias, características temporais do canto não mostraram qualquer tipo de limitação, com exceção de SR em Thamnophilidae que foi afetada pela morfologia do bico e massa corporal; (2) em geral os modelos mais simples, sem estrutura de correlação (White Noise) ou com uma taxa de evolução constante ao longo dos ramos da filogenia (Brownian motion) apresentaram melhor ajuste às características do canto, indicando que elas podem evoluir de modo mais simples do o usualmente imaginado; (3) massa corporal é uma importante característica que limita propriedades dos cantos e deveria ser incluída em qualquer estudo bioacústico comparativo; (4) a história evolutiva das famílias irá determinar o potencial para a evolução do canto e as características morfológicas e

fisiológicas impostas pela filogenia são importantes em limitar as propriedades acústicas dos cantos.

ABSTRACT

Acoustic communication has been a hallmark of avian evolution. Studies on birdsong evolution generally invoke the role of morphological characteristics influencing the sound-producing apparatus, habitat structure causing barriers on sound transmition, niche acoustic partitioning among species to avoid overlapping their signals and cultural drift during song learning process. The increasing data availability on the phylogenetic relationships between species, ecological information and the availability of high quality recordings allows for investigating how these factors contribute in shaping song evolution into a macroecological perspective. In this study we used phylogenetic comparative methods and spatial statistic to test classical constraints hypotheses (environmental and morphological) on song structure of suboscine (families Thamnophilidae, Tyrannidae and Pipridae) and oscine (families Parulidae and Turdidae) birds. In the first chapter we tested the Acoustic Adaptation Hypotheses (AAH) (environmental constraint) in a broad scale using a quantitative measure of vegetation, the Normalized Difference Vegetation Index (NDVI). After controlling the phylogenetic and spatial effects on song parameters, only Thamnophilidae and Tyrannidae exhibited the expected pattern under AAH related to song frequency, with a negative relation between song frequency and NDVI: songs in forest environment presented lower frequency values than songs in open environment. Most models with significant results included body mass, indicating the strong influence of morphology as a constraint on song frequency. On the other hand, temporal song traits analyzed not were affected by vegetation. In the second chapter we summarized the interspecific variation in song traits in these families using principal component analysis fitting alternative models of evolution (White noise, Brownian motion, Ornstein-Uhlenbeck and Early burst) on the PC scores. The first PC, which accounted for approximately 40% of the variance in all families, indicated that the main axis of birdsong evolution involves changes in song frequency. In addition, the simplest models (White noise and Brownian motion) showed the best fit to most of song principal component scores. Finally, in the third chapter we tested whether these families experience the tradeoff between frequency bandwidth (FB) and song rate (SR) already demonstrated in other taxa. We used upper bound and quantile regressions to estimate these relations. Upper bound results were affected by intervals established in song rate. Using 1 Hz only Pipridae exhibited the expected negative relation between FB and SR, but using 2 Hz, Thamnophilidae, Pipridae and Turdidae showed significant results. Given that upper bound and quantile regression showed conflicting results, we used phylogenetic generalized least squares (PGLS) analysis to test the effect of the beak size and body mass (morphological constraint) directly on FB and SR. Four beak measures were summarized using principal component analysis and the beak PC scores were posteriorly used in the PGLS analysis. In four families all beak measures were summarized into the first PC, only in Pipridae were needed two PCs to represent beak size. The best fit of PGLS models were that included the interaction between beak PC's and log of the body mass. Beak morphology and body mass affected FB and SR in Thamnophilidae and Parulidae. In Thamnophilidae, small beaks and body mass produces the faster rates and broad frequency bandwidth, consistent with the hypothesis of constraints on sound production. In conclusion, the results of this thesis provide evidence for the following general principles regarding birdsong evolution: (1) frequency characteristics are constrained by environment and morphology. However, to most families temporal song characteristics not showed any kind of constraint, with exception of song rate in antbirds that was affected by beak morphology and body mass; (2) in general simplest models representing no correlation structure (White noise) or a constant rate of evolution along the phylogeny branch (Brownian motion) presented the best fit to song characteristics, indicating that they could has evolved by more simple than usually is suspected; (3) body mass is a morphological characteristic extremely important in constraining song properties and should be included in any comparative study in bioacoustic, and finally (4) the evolutionary history of the families will determine the potential to song evolution and the morphological and physiological characteristics imposed by phylogeny are important in constraint acoustic song properties.

1. INTRODUÇÃO GERAL

O canto em aves é um caráter com considerável variação temporal e espacial e, devido ao seu papel na atração de fêmeas e defesa do território, está fortemente sujeito seleção natural e sexual (Searcy & Anderson 1986, Catchpole & Slater 1995, Badyaev & Leaf 1997, Slater 1989, Podos *et al*. 2004). A maioria dos estudos de vocalizações de aves envolve espécies da Ordem Passeriformes, um grupo monofilético subdividido nas subordens suboscines e oscines (Raikow 1982, Sick 1997). Na primeira, o canto é considerado inato, enquanto na segunda é o resultado de uma complexa interação entre genética e aprendizado (Baker & Cunningham 1985). Essa diferença entre as subordens tem implicações importantes: estudos com suboscines predominam na região tropical - onde há maior riqueza de espécies dessas aves e enfatizam o papel de barreiras geográficas (Cohn-Haft 2000), os limites de distribuição de espécies simpátricas (Payne 1986, Isler *et al*. 1998, Seddon 2007) e a influência do ambiente acústico na variação do canto (Lindel 1996, Seddon 2005, Tobias *et al.* 2010). Por outro lado, o número de espécies de oscines é bem maior em regiões temperadas, sendo que nesses locais predominam estudos sobre programas de aprendizagem (Kroodsma 1977, Nelson 1995, Payne 1996, Beecher & Brenowitz 2005) e variação geográfica no canto, conhecida como dialeto (Lemon 1967, Baptista 1977, Bitterbaum & Baptista 1979, Petrinovich & Patterson 1981, Podos & Warren 2007).

Do mesmo modo que qualquer outro fenótipo o canto está sujeito a diversas limitações que impossibilitam sua variação em qualquer direção. Dentre as limitações que afetam a evolução do canto estão a morfologia, o ambiente e a própria historia compartilhada entre as espécies. A estrutura da siringe, os núcleos cerebrais de controle produção do canto, a massa corporal e a forma e tamanho do bico são exemplos de características morfológicas que afetam o canto (Margoliash *et al.* 1994, Podos *et al.* 2004). Nas aves Oscines, a estrutura da siringe é composta por seis pares de músculos sob controle do ajuste da tensão motora muscular, sendo responsável pela modulação da freqüência fundamental do canto (Amador *et al.* 2008). No cérebro há um sistema especializado responsável pelo controle do canto, conhecido como "aparelho sonoro", formado por um circuito que inclui o alto centro de controle vocal e o núcleo robusto do arqueoestriado, ambos essenciais para a produção de cantos ao longo da vida, e também pelo circuito cerebral anterior, que é importante para o desenvolvimento do canto e plasticidade no aprendizado (Bottjer *et al*. 1984, Scharff & Nottebohm 1991, Marler & Doupe 2000, Farries 2004).

Nos Suboscines, existem de três a quatro pares de músculos siringeais e não há evidências de controle muscular para modulação do som, pelo menos nas poucas espécies estudadas (Nottebohm 1980, Kroodsma 1984, Kroodsma & Konishi 1991, Amador *et al.* 2008). Porém é desconhecido se esta aparente falta de circuito de controle motor do canto está relacionada à capacidade de controle vocal menos sofisticada desta subordem. Além disso, estudos sobre a estrutura cerebral deste grupo indicam que eles não apresentam centros responsáveis por mecanismos de aprendizado como os existentes em oscines (Brenowitz & Kroodsma 1996). Como resultado, o canto em suboscines é relativamente mais simples e estereotipado (Seddon 2007). Por exemplo, em Thamnophilidae foi demonstrado que a variação vocal exibe padrão geográfico e que o canto deve ser um caráter herdado e não aprendido (Brumfield 2005, Isler *et al*. 2005, Remsen 2005).

O tamanho corporal, estimado geralmente pela massa corporal das aves, está diretamente ligado ao tamanho da siringe e negativamente relacionado à freqüência, ou seja, aves maiores tendem a cantar em freqüências mais baixas. Esse padrão já foi demonstrado tanto em não Passeriformes (Bertelli & Tubaro 2002) como em Passeriformes (Wallchlager 1980). Ryan & Brenowitz (1985) demonstraram que há diferença na massa corporal entre aves de ambientes abertos e fechados e concluíram que estudos relacionando a freqüência do canto somente ao ambiente desconsiderando a massa corporal poderiam cometer equívocos.

A forma e o tamanho do bico também afetam a produção do canto, pois influenciam na capacidade de abertura e fechamento do bico no momento em que a ave canta, direcionando o desempenho e habilidade vocal, podendo inclusive modificar a estrutura do canto (Podos & Nowicki 2004). Nos tentilhões de Darwin, indivíduos maiores e com bicos mais robustos produzem cantos com menor taxa de repetição silábica e bandas de freqüência mais estreitas (Podos 2001). Essa relação entre morfologia do bico e estrutura do canto tem sido testada em uma variedade de espécies, com foco predominante em aves oscines (Ballentine 2006, Huber & Podos 2006, Derryberry 2009, Podos *et al.* 2009, Cardoso & Hu 2011, Wilson *et al.* 2014). Recentemente a hipótese de limitação morfológica sobre a estruturação do canto também foi testada em suboscines (Seddon 2005, Palacios & Tubaro 2000, Derryberry *et al.* 2012). Entretanto, os estudos mais antigos foram realizados com uma ou poucas espécies, e até hoje nenhum estudo comparativo foi realizado investigando os padrões entre aves Oscines e Suboscines. Essa comparação tem um potencial elucidativo, uma vez que as duas subordens representam linhagens divergentes com ampla variação morfológica e portanto, poderiam apresentar diferenças em como essas limitações morfológicas afetam os cantos.

Além da morfologia, o ambiente é capaz de exercer pressão seletiva sobre os cantos, direcionando sua evolução (Barker 2008, Brumm & Naguib 2009). Segundo a Hipótese de Adaptação acústica (HAA), (Morton 1975) os cantos são estruturados para maximizar seu desempenho sob as barreiras do ambiente acústico. Dessa forma, cantos de baixa freqüência, com pouca repetição de notas e presença de assovios são favorecidos em ambientes florestais, enquanto cantos de alta freqüência, com presença de elementos repetitivos e trinados são mais eficientes em ambientes abertos (Morton 1975, Hansen 1979, Wiley & Richards 1982, Rothstein & Fleischer 1987, Tubaro & Segura 1994, Brown & Handford 1996, Badyaev & Leaf 1997; Doutrelant *et al.* 1999).

Apesar de uma grande quantidade de estudos testando a HAA desde a década de 70, os resultados são frequentemente contraditórios, dependendo da espécie estudada e da escala geográfica do estudo. Enquanto alguns estudos suportam algumas das predições da hipótese (Badyaev & Leaf 1997; Bertelli & Tubaro 2002; Slabbekoorn *et al.* 2002; Slabbekoorn & Smith 2002; Patten *et al.* 2004; Nicholls & Goldizen 2006, Derryberry 2009, Kirschel *et al.* 2009), outros encontraram evidências opostas (Lemon *et al.* 1981; Daniel & Blumstein 1998, Tubaro & Mahler 1998, Blumstein & Turner 2005, Boncoraglio & Saino 2007). A maneira usual de testar a HHA é classificar a vegetação qualitativamente em "aberta" e "fechada", ou algumas vezes estabelecendo mais categorias para descrever as diferenças na fisionomia da vegetação. Possivelmente as diferenças entre os resultados desses estudos podem estar relacionadas a essa classificação subjetiva da vegetação.

Recentemente estudos sobre a evolução do canto vêm utilizando grandes conjuntos de dados e métodos filogenéticos comparativos para controlar o efeito das relações filogenéticas entre os fatores investigados, e as questões que antes se restringiam a uma espécie ou população agora são direcionadas para padrões em larga escala. Por exemplo, Weir & Wheatcroft (2011) observaram que existe um gradiente latitudinal na diversidade de sílabas e comprimento do canto entre aves oscines e suboscines. Em latitudes mais altas a taxa de evolução do canto em aves oscines é vinte vezes mais rápida do que em regiões tropicais, sugerindo que taxas evolutivas em características tão importantes como o canto são influenciadas pela latitude e, ao contrário do esperado, essas taxas são maiores em regiões temperadas onde a diversidade de espécies é menor.

O padrão de variação na freqüência do canto também foi analisado em escala macrogeográfica, mostrando que espécies em regiões tropicais em ambiente florestal cantam em freqüências mais baixas e utilizam uma variação menor nas faixas de freqüência do que as espécies de ambientes temperados. Esse resultado sugere a existência de uma janela acústica menor nos trópicos devido à presença de insetos que sinalizam em algumas faixas de freqüência específica e que competiriam com os cantos das aves (Weir *et al*. 2012). Além disso, modelos de evolução foram ajustados à freqüência dos cantos, indicando limitação diferenciada em ambiente tropical e temperado, sendo que a frequência dos cantos evolui mais rápido em ambiente temperado independentemente do tipo de habitat (floresta ou vegetação aberta). Outro estudo comparativo recente testando a HAA mostrou que em Thraupidae a taxa de mudança na frequência, a distribuição de frequência das notas e a taxa de repetição de elementos foram consistentes com as predições da hipótese, enquanto outros parâmetros de freqüência do canto como freqüência de pico, freqüência maior e menor variaram em direção oposta a daquela predita pela HAA (Mason 2012).

Todos os estudos mencionados acima trazem contribuições importantes para o entendimento da evolução do canto, entretanto, nenhum deles investigou a contribuição das diferentes limitações (ambiental, filogenética e morfológica) sobre a evolução do canto ao mesmo tempo e em diferentes linhagens utilizando uma ampla escala geográfica. Portanto, este estudo tem por objetivo investigar a contribuição do ambiente, filogenia e morfologia sobre a evolução do canto de aves suboscines (Thamnophilidae, Tyrannidae e Pipridae) e oscines (Parulidae e Turdidae) em escala macroecológica. A escolha das famílias do presente estudo foi baseada na disponibilidade de filogenias moleculares publicadas, cantos de boa qualidade depositados em coleções, disponibilidade de mapas de distribuição das espécies georeferenciados e peles em museus. Além disso, as famílias foram selecionadas buscando uma ampla variação em relação à morfologia, comportamento e distribuição geográfica a fim de testar a generalidade dessas hipóteses de limitações sobre a estruturação do canto em larga escala. O presente estudo foi estruturado em três capítulos. No primeiro capítulo foram utilizados métodos filogenéticos comparativos e estatística espacial a fim de testar a hipótese de adaptação acústica em escala macrogeográfica (Américas), utilizando uma medida quantitativa de vegetação, o Normalized Difference Vegetation Index (NDVI), com o objetivo de entender o papel do ambiente na evolução do canto. No segundo capítulo foram ajustados diferentes modelos de evolução aos parâmetros do canto das famílias estudadas, procurando compreender como a filogenia contribui para a evolução dessas características. E finalmente, no terceiro capítulo foi testada a generalidade da hipótese de limitação morfológica sobre a estrutura dos cantos, comparando os padrões encontrados entre aves Oscines e Suboscines.

2. OBJETIVO GERAL

Realizar um estudo comparativo e abrangente de como a filogenia, morfologia e o ambiente moldaram a evolução do canto em aves oscines e suboscines em ambientes temperados e tropicais.

2.1. OBJETIVOS ESPECÍFICOS

i. Testar a Hipótese de Adaptação Acústica em escala macrogeográfica e mapear os padrões geográficos dos parâmetros dos cantos.
ii. Testar o ajuste de diferentes modelos de evolução para os diferentes parâmetros dos cantos.
iii. Testar se a massa corporal e o tamanho do bico afetam a estrutura dos cantos (Hipótese de restrição morfológica).

REFERÊNCIAS

Amador, A.; Goller, F.; Mindlin, G. B. 2008. Frequency Modulation During Song in a Suboscine Does Not Require Vocal Muscles. *Journal of Neurophysiology* 99: 2383-2389.

Badyaev, A.V.; Leaf, E. S. 1997. Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* Warblers. *Auk*, 114: 40-46.

Baker, M. C. 1981. Early experience determines song dialect responsiveness of female sparrows. *Science* 214: 819-820.

Baptista, L. F. 1977. Geographic variation in song and dialects of the Puget sound white – crowned sparrow. *Condor* 79: 356-370.

Barker, N. 2008. Bird song structure and transmission in the Neotropics: trends, methods, and future directions. *Ornitologia Neotropical* 19: 175-199.

Beecher, M. D.; Brenowitz, E. A. 2005. Functional aspects of song learning in songbirds. *Trends in Ecology and Evolution* 20: 143-149.

Bertelli, S.; Tubaro, P. L. 2002. Body mass and habitat correlates of song structure in a primitive group of birds. Biological Journal of the Linnean Society 77: 423-430.

Bitterbaum, E.; Baptista, L. F. 1979. Geographical variation in songs of California House Finches (*Carpodarcus mexicanus*). *Auk* 96: 462-474.

Boncoraglio, G.; Saino, N. 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology* 21: 134-142.

Bottjer, S.W.; Miesner, E.A.; Arnold AP. 1984. Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science* 224: 901-903.

Brown, T. J.; Handford, P. 1996 Acoustical signal amplitude patterns: a computer simulation investigation of the acoustic adaptation hypothesis. *Condor* 98: 608-623.

Brumm, H.; Naguib, M. 2009. Environmental Acoustics and the Evolution of Bird Song. *Advances in the Study of Behavior* 40: 1-33.

Brenowitz, E.A.; Kroodsma, D.E., 1996. The neuroethology of birdsong. In: Kroodsma, D.E., Miller, E.H. (Eds.), Ecology and Evolution of Acoustic Communication in Birds. Cornell University Press, Ithaca, NY, pp. 285-304.

Brumfield, R.T. 2005. Mitochondrial variation in Bolivian populations of the variable antshrike (*Thamnophilus caerulescens*). *Auk* 122: 414-432.

Cardoso, G. C.; Hu, Y. 2011. Birdsong Performance and the Evolution of Simple (Rather than Elaborate) Sexual Signals. *American Naturalist* 178: 679-686.

Catchpole, C. K.; Slater, P. J. B. 1995. *Bird Song: biological themes and variation*. Cambridge University Press. 185-218.

Cohn-Haft, M. 2000. A case study in amazonian biogeography: vocal and DNA-sequence variation in *Hemitriccus* flycatchers. Ph.D. diss. Louisiana State University, Baton Rouge, EUA.

Daniel, J. C.; Blumstein, D. T. 1998. A test of the acoustic adaptation hypothesis in four species of marmots. *Animal Behaviour* 56: 1517-1528.

Derryberry, E. 2009. Ecology Shapes Birdsong Evolution: Variation in Morphology and Habitat Explains Variation in White-Crowned Sparrow Song. *American Naturalist* 174: 24-33.

Derryberry, E.P.; Seddon, N.; Claramunt, S.; Tobias, J. A.; Baker, A.; Aleixo, A.; Brumfield, R. T. 2012.Correlated evolution of beak morphology and song in the Neotropical woodcreper radiation. *Evolution* 66: 2784-2797.

Doutrelant, C.; Leitão, A. Georg, M.; Lambrechts, M. M. 1999. Geographic variation in blue tit songs: the result of an adjustment to vegetation type? *Behavioral Science* 136: 481-493.

Farries, M. A. 2004.The Avian Song System in Comparative Perspective. *Annals of the New York Academy of Sciences* 1016: 61-76.

Hansen, P. 1979: Vocal learning: its role in adapting sound structures to long-distance propagation and a hypothesis on its evolution. *Animal Behaviour* 27: 1270-1271.

Huber, S. K.; Podos, J. 2006. Beak morphology and song features covary in a population of Darwin's finches (*Geospiza fortis*). *Biological Journal of the Linnean Society* 88: 489-498.

Isler, M.; Isler, P. R.; Whitney, B. M. 1998. Use of vocalizations to establish species limits in antbirds (Passeriformes: Thamnophilidae). *Auk* 115: 577-590.

Isler, M.L.; Isler P.R.; Whitney, B.M. 2005. Clinal variation in vocalizations of an antbird (Thamnophilidae) and implications for defining species limits. *Auk* 122: 433-444.

Kroodsma, D. E.1984. Song of the alder flycatcher (*Empidonax alnorum*) and willow flycatcher (*Empidonax trailli*) are innate. *Auk* 96: 506-515.

Kirschel, A. N. G; Blumstein, D. T.; Cohen, R. C.; Buermann, W.; Smith,T. B.; Slabbekoorn, H. 2009. Birdsong tuned to the environment: green hylia song varies with elevation, tree cover, and noise. *Behavioral Ecology* 20:1089-1095.

Kroodsma, D. E.; Konishi, M. 1991. A suboscine bird (eastern phoebe, Sayornis phoebe) develops normal song without auditory feedback. *Animal Behaviour* 42: 477-487.

Kroodsma, D. E. 1977. Correlates of song organization among North American wrens. *American Naturalist* 111: 995-1008.

Lemon, R. E. 1967. The response of cardinals to songs of different dialects. *Animal Behaviour* 15: 538-545.

Lindell, C. 1998. Limited geographic variation in the vocalizations of a neotropical Furnariid, *Synallaxis albescens*. *Wilson Bulletin* 110: 368-374.

Margoliash, D.; Fortune, E. S.; Sutter, M. L.; Yu, A. C.;Wren-Hardi, D. B; Dave, A. 1994.Distributed Representation in the Song System of Oscines: Evolutionary lmplications and Functional Consequences. *Brain Behavior and Evolution* 44: 247-264.

Marler, P.; Doupe, J. A. 2000. Singing in the brain. *PNAS* 97: 2965-2967.

Mason, Nicholas. 2012. Song complexity and its evolutionary correlates across a continent-wide radiation of songbirds. MSc thesis, San Diego State University.

Morton, E. S. 1975. Ecological sources of selection on avian sounds. *American Naturalist* 109: 17-34.

Nelson, D. A. 1995. A comparative approach to vocal learning: intraspecific variation in the learning process. *Animal Behaviour* 50: 83-97.

Nicholls, J. A.; Goldizen, A. W. 2006. Habitat type and density influence vocal signal design in satin bowerbirds. *Journal of Animal Ecology* 75: 549-558.

Nottebohm F. 1980. Brain pathways for vocal learning in birds: a review of the first 10 years. *Progress Psychobiology and Physiological Psychology* 9: 85-124.

Palacios, M. G.; Tubaro, P. L. 2000. Does beak size affect acoustic frequencies in Woodcreepers? *Condor* 102: 553-560.

Payne, R. B. 1996. Song Traditions in Indigo Buntings: origin, improvisation, dispersal and extinction in cultural evolution. *In: Ecology and Evolution of Acoustic Communication* *in Birds* Eds: Kroodsma, D. E. and Miller, E. H. Cornell University Press. Ithaca and London.

Payne, R. B. 1986. Bird Song and avian systematics. *Current Ornithology* (3). Ed.Richard F. Johnston.Plenum Press, New York and London. 87-126.

Petrinovich, L.; Patterson, T. L. 1981. The responses of white-crowned sparrows to songs of different dialects and subspecies. *Zeitschrift Fur Tierpsychologie* 57: 1-14.

Podos, J.; Lahti, D. C.; Moseley, D. L. 2009. Vocal performance and Sensorimotor learning in songbirds. *Advances in the Study of Behavior* 40: 159-195.

Podos, J.; Warren, P.S. 2007. The evolution of geographic variation in birdsong *Advances in the Study of Behavior* 37: 403-458.

Podos, J.; Nowicki, S. 2004. Beaks, Adaptation, and Vocal Evolution in Darwin's Finches *Bioscience* 54 (6): 501-510.

Podos, J.; Huber, S. K.; Taft, B. 2004. Bird Song: The Interface of Evolution and mechanism. *Annual Review of Ecology, Evolution and Systematics* 35: 55-87.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409: 185-188.

Raikow, R. J. 1982. Monophily of the Passeriformes: test of a phylogenetic hypothesis. *Auk* 99: 431-445.

Remsen, J. V. 2005. Pattern, process and rigor meet classification. *Auk* 122: 403–413.

Richards, D. G.; Wiley, R. H. 1980. Reverberations and amplitude fluctuations in the propagation of sound in a forest: implications for Animal communication. *American Naturalist* 115: 381-399.

Rothstein, S. I.; Fleischer, R. C. 1987. Vocal dialects and their possible relation to honest status signaling in the brown-headed cowbird. *Condor* 89: 1-23.

Ryan, M. J.; Brenowitz, E. A. 1985. The role of Body Size, Phylogeny and Ambient Noise in the evolution of Bird Song. *American Naturalist* 126: 87-100.

Scharff, C.; Nottebohm, F .1991. A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. *Journal of Neuroscience* 11: 2896-2913.

Searcy, W. A.; K. Yasukawa. 1996. Song and female choice. Pp. 454-473 in D. E. Kroodsma and E. H. Miller, eds. *Ecology and evolution of acoustic communication in birds*. Cornell Univ.Press, Ithaca, NY.

Searcy, W. A.; Anderson, M. 1986. Sexual selection and the evolution of song *Annual Review of Ecology, Evolution and Systematics* 17**:** 507-533.

Seddon, N.; Tobias, J. A. 2007. Song divergence at the edge of Amazonia: an empirical test of the peripatric speciation model. *Biological Journal of the Linnean Society* 90: 173-188.

Seddon, N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution* 59: 200-215.

Sick, H. 1997. *Ornitologia Brasileira*. Ed. Nova Fronteira: 692-718.

Slabbekoorn, H.; Smith, T. B. 2002. Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society B* 357: 493-503.

Slabbekoorn, H.; Ellers, J.; Smith, T. B. 2002. Birdsong and sound reverberation: the benefits of reverberations. *Condor* 104: 564.

Slater, P. J. B. 1989. Bird song learning: causes and consequences. *Ethology Ecology & Evolution* 1: 19-46.

Tobias, J. A.; Aben, J.; Brumfield, R. T.; Derryberry, E. P.; Halfwerk, W.; Slabbekoorn, H.; Seddon, N. 2010. Song Divergence by Sensory Drive in Amazonian Birds. *Evolution* 64: 1-20.

Tubaro, P. L.; Mahler, B. 1998. Acoustic frequencies and body mass in New World doves. *Condor* 100: 54-61.

Tubaro, P. L.; Segura, E. T. 1994. Dialect differences in the song of *Zonotrichia capensis* in the southern Pampas: a test of the acoustic adaptation hypothesis. *Condor* 96: 1084-1088.

Wallschlager. D. 1980. Correlation of song frequency and body weight in passerine birds *Experientia* 36: 412.

Weir, J. T.; Wheatcroft, D.; Price. T. 2012. The role of ecological constraint in driving the evolution of avian song frequency across a latitudinal gradient. *Evolution* 66: 2773-2783.

Weir, J.; Wheatcroft, D . 2011. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Proceedings of Royal Society B* 278: 1713-1720.

Wiley, R. H.; Richards, D. G. 1982. Adaptations for acoustic communication in birds: sound transmission and signal detection. *In: Acoustic Communication in Birds* (Kroodsma, D. E. & Miller, E. H., eds). Academic Press, New York & London: 131-181.

Wilson, D. R.; Bitton, P. B.; Podos, J.; Mennill, D. J. 2014. Uneven Sampling and the Analysis of Vocal Performance Constraints. *American Naturalist* 183: 214-228.

3. ARTIGO I

A macroecological test of the acoustic adaptation hypothesis

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A macroecological test of the acoustic adaptation hypothesis

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Running head: Testing the acoustic adaptation hypothesis.

3.1. Abstract

One of the most important hypotheses on song evolution is the acoustic adaptation hypothesis (AAH), which posits that environmental pressures act on species by shaping their songs. In dense forests, selection would favour longer songs, with lower frequencies, and fewer note repetitions or longer note duration, with the opposite pattern being favoured in open environments. A large number of studies have tested these predictions, yet most were conducted with only one or few species, and with the exception of two large-scale studies, their macroecological patterns remain unknown. In this study, we tested the AAH predictions in a broad geographical scale using a quantitative measure of the vegetation (NDVI), and controlling the phylogenetic and spatial effects on songs parameters of three Suboscines families (Thamnophilidae, Tyrannidae and Pipridae) and two Oscines families (Parulidae and Turdidae). After controlling for the phylogenetic and morphological constraints on song parameters, only Thamnophilidae and Tyrannidae exhibited the expected pattern under AAH related to song frequency, showing a negative relation between song frequency and NDVI. Most models with significant results included body size, indicating the strong influence of morphology as a constraint on song frequency. Our results highlight two main implications: frequency seems to be more constrained by physical characteristics of the environment than temporal song parameters and the influence of the environment in constraining birdsong might not be a widespread phenomenon in bird song evolution, at least at large geographical scales. Possibly, morphological and ecological differences among these families respond differently to environmental constraints.

3.2. Introduction

Acoustic communication has been one of the major hallmarks of bird evolution (Kroodsma & Miller 1996). The remarkable variety of sounds generated during bird vocalization as a consequence of the advent of the syrinx allowed birds to produce a bewildering diversity of songs of varying levels of complexity (Fagerlund 2004; Suthers & Zollinger 2004). Although their function is most commonly associated with sexual selection (Slater 1989; Price 1998; Nowicki & Searcy 2004), bird vocalizations might also play important roles in a variety of contexts, including defence (e.g. mobbing and alarm calls: Catchpole & Slater 2008), communication with nestlings (Leonard & Horn 2001; Marques *et al.* 2009) and even echolocation (Suthers & Hector 1982; 1985).

Passerines (order Passeriformes) include the highest diversity of both species and songs among all birds (Marler & Slabbekoorn 2004). Interestingly, the earliest split during the evolution of passerines was marked by the evolution of two fundamentally different modes of song evolution. Oscines are mostly temperate birds with developed syringeal muscles (Amador *et al.* 2008) and are able to learn songs, thus displaying higher song plasticity (Baker & Cunningham 1985). On the other hand, Suboscines, which are most common in the tropics, usually possess a simpler syrinx structure and display more stereotyped song patterns (Kroodsma & Konishi 1991; Isler *et al.* 2005, Amador *et al.* 2008; Seddon & Tobias 2007). Most studies to date have focused on Oscines, including topics such as learning programs (Kroodsma 1977; Nelson 1992; Podos *et al.* 1999, Beecher & Brenowitz 2005; Nulty *et al.* 2010) and song geographic variations, known as dialects (Lemon 1967; Bitterbaum & Baptista 1979; Cunningham *et al.*1987; Podos & Warren 2007; Liu *et al.* 2008), whereas suboscine songs have been comparatively less studied.

One of the most general hypotheses about the forces driving the song evolution is the acoustic adaptation hypothesis (AAH), which provides specific predictions as to how the acoustic environment would shape bird song characteristics (Morton 1975). For instance, sound transmission can suffer interference from elements of the soil or the vegetation, and some sound frequencies can experience attenuation by wind, humidity, and temperature (Richards & Wiley 1980; Wiley & Richards 1982). As a consequence, natural selection on forest bird species would favour longer songs, with lower frequencies and fewer note repetitions. In contrast, open environments would favour shorter songs, with higher frequencies and more frequent repetitions (Wiley 1991; Doutrelant *et al.* 1999; Barker 2008; Tobias *et al.* 2010). Despite these clear predictions and the large number of studies testing the AAH, results to date have been often contradictory, depending on the studied species and study scale. Although many studies confirmed some of the hypothesis predictions (Badyaev & Leaf 1997; Bertelli & Tubaro 2002; Slabbekoorn *et al.* 2002; Slabbekoorn & Smith 2002; Nicholls & Goldizen 2006; Derryberry 2009; Kirschel *et al.* 2009), other found conflicting evidence (Lemon *et al.* 1981; Daniel & Blumstein 1998; Tubaro & Mahler 1998; Blumstein & Turner 2005; Boncoraglio & Saino 2007). Possibly, some of these discrepancies might be due to the subjective classification of vegetation physiognomies and the local scale used in these studies, such as the classification of vegetation as either "open" or "closed". Here, instead this classification, we used the Normalized Difference Vegetation Index (NDVI), which is a measure of photosynthetically active green biomass: higher values represent more living green biomass (Vinciková *et al.*2010). This index is a more precise measure of plant biomass and therefore should allow for more precise inferences regarding AAH.

The field of macroecology has revolutionized the study of a variety of long-standing ecological issues, as the latitudinal gradient in species richness (Hawkins *et al.* 2007), Bergmann's rule (Diniz-Filho *et al*. 2007; Ollala-Tárraga *et al.* 2009; Cooper & Purvis 2010) and, more recently, phylogenetic niche conservatism (Hawkins *et al.* 2006; Wiens *et al.*2006; Rangel *et al.*2007; Ramirez *et al.*2008; Kerkhoff *et al*. 2014). However, little is known about the macroecological pattern of phenotypic traits. Investigating how bioacoustic features of bird songs are distributed over large spatial scales can provide a valuable tool for understanding the evolutionary dynamics of these traits (Weir & Wheatcroft 2011; Cardoso & Hu 2011; Weir *et al.* 2012). In this study, we test the AAH in a broad geographical scale (Americas), among Suboscine (Thamnophilidae, Tyrannidae and Pipridae) and Oscine bird families (Turdidae and Parulidae). In particular, we use phylogenetic comparative methods and spatial statistics to integrate information on song acoustic properties of a representative sample of these families and GIS-based information to test the AAH in a macroecological perspective and mapping song parameters.

3.3. Methods

Song measurements

Recordings were obtained from the following collections: Instituto de Investigación de Recursos Biológicos Alexander Von Humboldt (Colombia), Acervo Neotropical Elias Coelho (Universidade Federal do Rio de Janeiro, Brazil), Macaulay Library (Cornell University, EUA), XENO-CANTO database (http://www.xeno-canto.org), and private song collections of Jeremy Minns and Christian Borges Andretti. We measured a total of 3173 recordings from suboscine (Thamnophilidae, Tyrannidae, and Pipridae) and oscine families (Parulidae and Turdidae) (Table 1). For suboscines we measured one phrase per individual, given this suborder shows stereotyped songs with little variation in repertoire. However, in oscines, due to learning, one individual can sing a large number of different phrases within a song. In this case, to capture the song variation throughout a species, we analysed all the different phrases sung by each individual within an interval of three minutes. Songs with sample rate and resolution less that 22.050 Hz and 16 bits were discarded. All the spectrograms were generated using the software AVISOFT SAS Lab Pro 5.1 (Specht 2011), with the following specifications: Window: Hamming, FFT: 256, Frame Size: 100%, and Overlap: 88%. We used the “two thresholds” automatic parameters with the threshold fitted to each song independently, visualizing the best cut-off value in the power spectrum graphic, which allows for selecting all notes while excluding the noise in the background. For each phrase we measured the following acoustic parameters: 1) mean phrase duration (SD): (s), the duration from the beginning of the first element (note) to the end of the last element in the phrase; 2) mean number of notes (NN): number of elements (notes) detected within the phrase; 3) element rate (ER): (s), the average duration of the elements (notes) within a phrase; 4) peak frequency (PF): (KHz), at maximum spectrum (peak hold) of the entire phrase. To measure the maximum and minimum frequencies (F_{max} and F_{min}, respectively), we used the manual cursor because songs show considerable variation in relation to the presence of harmonics, which hamper the accurate automatic estimation of these parameters. Frequency bandwidth (FB) was obtained by subtracting F_{min} from F_{max}. We used species means for each measurement in further analyses.

Phylogenetic and Spatial Analysis

Predictions of the AAH were tested by assessing the correspondence between the level of vegetation cover found throughout the distribution of each species (as measured by

NDVI) and its song acoustic properties. This assessment involved four main steps. First, we obtained the distribution shapefiles of the species from the NATURESERVE database (http://www.natureserve.com). In ARCGIS 9.3 (ESRI 2008) we generated a set of random coordinates within the distribution of each species using the "GENERATE RANDOM POINTS TOOL" (Beyer, H. L. 2004. Hawth's Analysis Tools for ArcGIS. Available at http://www.spatialecology.com/htools). We used the point density equal to 10, such that the number of simulated coordinates was proportional to the size of the distribution of the species. From the NDVI raster (obtained from the Center for Satellite Applications and Research, available at http://www.star.nesdis.noaa.gov) we extracted values from each simulated coordinate and posteriorly calculated an average within-species NDVI.

Finally, the influence of phylogenetic relationships, NDVI and acoustic characteristics were tested using the method of Freckleton & Jetz (2009) in R, 3.0.1 (R Development Core Team, 2014). This method is based on the estimation of the parameters λ' and φ, which vary from 0 to 1 and reflect the extent of phylogenetic or spatial autocorrelation, respectively. We fitted different models to each family and estimated λ' and φ, based on the following phylogenies: Thamnophilidae (Gomez *et al*. 2010), Tyrannidae (Ohlson *et al.* 2008), Pipridae (Ohlson *et al.* 2008), Parulidae (Lovette *et al.* 2010) and Turdidae (Klicka *et al.* 2005). Given that body size might also influence the acoustic properties of songs (Wallschläger 1980; Ryan & Brenowitz 1985; Wiley 1991; Bertelli &Tubaro 2002), we used the logarithm of body mass (in g) as a covariate in all tested models. We obtained information about body mass from Handbook of the Birds of the World (del Hoyo *et al.* 2003; 2004; 2005 and 2010) and from Dunning (2008), using median values when only ranges were reported or when masses were reported separately for males and females. We used ARCGIS 9.3, establishing a grid on the species distribution with cells of 0.5 degrees and then we mapped the residuals of the regressions between the average of the song parameters and species richness in each cell. This procedure was made because cells with more species showed disproportionally higher values to song parameters when these were directly mapped. If song parameters support the AAH, then we expect that the maps these residuals and NDVI will exhibit similar patterns.

3.4. Results

There was no consistent pattern of phylogenetic or spatial autocorrelation among the tested song traits among families (Table 2). Of all 40 performed analyses (eight song parameters from five families), half of the tested traits showed independence with respect to both space and phylogeny, followed by 14 cases of predominantly phylogenetic autocorrelation, and 6 cases of substantial spatial autocorrelation. On the other hand, families differed substantially regarding the estimated values of λ'and φ. The families Thamnophilidae and Parulidae showed a predominance of phylogenetic autocorrelation (6 and 4 out of 8 tested traits, respectively). In particular, song acoustic characteristics in Tyrannidae, Pipridae, and Turdidae were generally independent of space and phylogenetic history, with higher γ values for 5, 6, and 5 out of 8 tested traits, respectively (Table 2).

Once potential spatial and phylogenetic autocorrelation were accounted for, the prediction of a relationship between song characteristics and NDVI was tested separately for each family and measured trait. In general, AAH predictions were not supported at the studied scale (Table 3). For instance, no significant association was found between NDVI and the variables SD, NN, and FB for any of the investigated families. In the case of Parulidae, no significant association was found between NDVI and any of the tested song variables. In Pipridae, the interaction between NDVI and body mass was significantly associated with PF and F_{max}, and yet for Turdidae, the interaction term was significant for PF and F_{min}. However, the direction of variation of the PF for Pipridae and Turdidae was the opposite of that expected by the AAH, with higher values being observed for more forested areas (Table 3, Fig 3 and 4, respectively). In contrast, in Thamnophilidae, PF, F_{max}, and F_{min} were significant and negatively associated with NDVI, as predicted by the AAH (Table 3, Fig. 1). Likewise, in Tyrannidae, results for F_{max} also corroborated the hypothesis, with lower frequency values being concentrated in regions of high NDVI values (Fig 2).

3.5. Discussion

This is the first study to investigate the AAH in a macroecological scale using tools to control simultaneously potential effects of phylogenetic and spatial autocorrelation. In particular, we tested whether birds living in forest regions presented longer songs, with lower frequencies, and higher note duration when compared to birds living in more open vegetation. We showed that, after controlling by phylogenetic and spatial effects (Table 2), most of song parameters analyzed were inconsistent with AAH predictions (Table 3). Interestingly, for all families included in the present study, only frequency parameters supported AAH, particularly in Thamnophilidae and Tyrannidae. In Pipridae and Turdidae, these relations also were significant, but in the opposite direction than that predicted by the hypothesis. These results suggest two main implications: first, frequency song parameters seem to be more constrained by physical characteristics of the environment than temporal song parameters and, second, the influence of the environment in constraining birdsong might not be a widespread phenomenon in shaping bird song evolution.

Song optimal structure to transmition depends on several factors acting together: typical communication distance, acoustic characteristics of the habitat, ambient noise profiles, and physical and phylogenetic constraints (Brumm & Naguib 2009). Therefore, complex patterns for the evolution of bird song are expected and our results reinforce these various observations in respect to the generality of AAH. For instance, in Thamnophilidae, the strong phylogenetic signal in most song parameters (Table 2) and the influence of the beak morphology and body mass on frequency and song rate (unpublished data, see Table 4 in Chapter 3 for more details) suggests that the contribution of the evolutionary history in this family is as important as physical characteristic of the environment in shaping song evolution. These results agree with those in a study by Seddon (2005) which analyzed 163 thamnophilid species testing predictions of morphological adaptation, acoustic adaptation, and the species recognition hypotheses. In that study, AAH was tested using strata as a proxy of vegetation structure. Species were assigned to different codes, according to the strata in which they habitually sang. Likewise, the AAH predictions related to frequency were supported: understory and canopy birds sing higher-pitched songs than birds living in the midstory, suggesting that song structure is related to the sound transmission properties of different habitat strata. Another study using local scale and small differences in vegetation physiognomies found similar results (Tobias *et al.* 2010), i.e. pairs of closely related Amazonian birds (some antbirds) occurring in bamboo and *Terra firme* forests were compared with the purpose of investigating whether vocal divergence between these two groups could be explained by ambient noise, correlated evolutionary response to beak and body size or genetic drift. Their results showed that song divergence was correlated with the sound transmission properties of the habitats, rather than with genetic divergence, ambient noise, or effects of mass, and beak size.

When considering Tyrannidae, Pipridae and Turdidae, the phylogenetic history did not represent an important constraint on song parameters, although songs in these taxa also have been affected by NDVI. In Tyrannidae, F_{max} followed the AAH predictions, with forest birds presenting lower F_{max} values than birds living in more open habitats. However, in Pipridae and Turdidae, frequency song parameters exhibited variation in an opposite direction than that predicted by AAH, and body mass was an important parameter in the model, once all significant results showed interaction between body mass and NDVI. We might speculate that, in Pipridae, sexual selection might be the main force driving song evolution. In this family, males display reproductive behavior in groups or "leks" in arenas that are located at the same place year after year. An adult male can spend more than 90% of daylight hours centered on the lek, with brief absences for foraging (Snow 2004). Thus, it seems reasonable that environmental constraint is not a strong pressure on song evolution in this family, given that vocalizations are simpler and sound transmition is assured by a reproductive strategy, in which males remain at a short distance from females, differently from other birds, in which males need to defend larger territories and cover longer distances to forage. In Turdidae, we believe that the lack of phylogenetic signal on frequency song parameters and the existence of learning could add sufficient plasticity in songs, allowing birds to change their frequency patterns, despite the environmental constraints, meaning that the cultural evolution and ontogenetic adaptation have an important role in shaping oscine song evolution (Brumm & Naguib 2009).

Despite an extensive literature about AAH, only recently the hypothesis' predictions have been investigated under a macroecological view (Mason 2012, Weir *et al.* 2012), and yet there are inconsistencies between studies. For instance, in the case of Thraupidae, frequency shift rates, average note bandwidths, and trill rates were consistent with AAH, whereas peak, high and minimum frequency parameters did not support the hypothesis (Mason 2012). On the other hand, another comparative study investigating the latitudinal pattern in song frequency showed that tropical latitudes constrained song frequency to low values and narrower bandwidths when compared to temperate latitudes (Weir *et al.* 2012).

When considered together, previous studies and present results reinforce that, despite differences among which song parameters are affected by the environment, AAH has a general support in relation to song frequency parameters, regardless of the study scale (small vs. broad scale), methods used to estimate differences in vegetation (qualitative vs. quantitative), or more detailed information about microhabitat (strata). However, it is also clear that considering only vegetation to test this hypothesis is not sufficient, because morphology is a factor of interference on song frequency (our results, Ryan & Brenowitz 1985). In this study we investigated whether the AAH predictions would be supported when using a quantitative measure of vegetation and controlling by phylogeny spatial effects and body size. We suggest that future research investigating AAH should also include information about strata favorably used by birds, given this information could add some important information about vegetation that could not have been captured by NDVI alone.

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4.6. References

Amador, A., Goller, F. & Mindlin, G. B. (2008) Frequency Modulation During Song in a Suboscine Does Not Require Vocal Muscles. *Journal of Neurophysiology*, **99**, 2383-2389.

Badyaev, A.V. & Leaf, E. S. (1997) Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* Warblers. *Auk*, **114**, 40-46.

Baker, M. C. & Cunningham, M. A. 1985 The biology of bird-song dialects. *Behavioral and Brain Sciences*, **8**, 85-133.

Barker, N. (2008) Bird song structure and transmission in the Neotropics: trends, methods, and future directions. *Ornitologia Neotropical*, **19**,175-199.

Beecher, M. D. & Brenowitz, E. A. (2005) Functional aspects of song learning in songbirds. *Trends in Ecology and Evolution*, **20**, 143-149.

Bertelli, S. &Tubaro, P. L. (2002) Body mass and habitat correlates of song structure in a primitive group of birds. *Biological Journal of the Linnean Society*, **77**, 423-430.

Bitterbaum, E. & Baptista, L. F. (1979) Geographical variation in songs of California House Finches (*Carpodarcus mexicanus*). *Auk*, **96**, 462-474.

Blumstein, D. T. & Turner, A. C. (2005) Can the acoustic adaptation hypothesis predict the structure of Australian birdsong? *Acta Ethologica*, **15**, 35-44.

Boncoraglio, G. & Saino, N. (2007) Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology*, **21**, 134-142.

Brumm, H. & Naguib, M. (2009) Environmental Acoustics and the Evolution of Bird Song. *Advances in the Study of Behavior*, **40**, 1-33.

Cardoso, G. C. & Hu, Y. (2011). Birdsong Performance and the Evolution of Simple (Rather than Elaborate) Sexual Signals. *American Naturalist*, **178**, 679-686.

Catchpole, C. K. & Slater, P. J. B. (2008) Variation in time and space. Bird Song: biological themes and variation, pp. 185-218. Cambridge University Press.

Collar, N. (2005) Family Turdidae (Thrushes). pp -514-807 in J. del Hoyo, A. Elliott and J. Sargatal, eds. Handbook of the Birds of the World, Vol. 10. Lynx Edicions, Barcelona.

Cooper, N. & Purvis, A. (2010) Body Size Evolution in Mammals: Complexity in Tempo and Mode. *American Naturalist*, **175**, 1-14.

Cunningham, M. A., Baker, M.C. & Boardman, T. J. (1987) Microgeographic song variation in the Nutall's White-Crowned sparrow. *Condor*, **89**, 261-275.

Curson, J. (2010) Family Parulidae (New World Warblers). pp. 666-800 in J. del Hoyo, A. Elliott and J. Sargatal, eds. Handbook of the Birds of the World, Vol.15. Lynx Edicions, Barcelona.

Daniel, J. C. & Blumstein, D. T. (1998) A test of the acoustic adaptation hypothesis in four species of marmots. *Animal Behaviour*, **56**, 1517-1528.

Derryberry, E. (2009) Ecology Shapes Birdsong Evolution: Variation in Morphology and Habitat Explains Variation in White-Crowned Sparrow Song. *American Naturalist,* **174**, 24-33.

Diniz-Filho, J. A. F., Bini, L. M., Rodriguez, M. A. Rangel, T. F. L. V. B. & Hawkins, B. A. (2007) Seeing the forest for the trees: partitioning ecological and phylogenetic components of Bergmann's rule in European Carnivora. *Ecography*, **30**, 598-608.

Doutrelant, C., Leitão, A. G. M. & Lambrechts, M. M. (1999) Geographic variation in blue tit songs: the result of an adjustment to vegetation type? *Behavioral Science*, **136**, 481-493.

Dunning, J. B. (2008). CRC Handbook of avian body masses. CRC Press, Taylor & Francis Group, Boca Raton, USA.

Fagerlund, S. (2004) Automatic Recognition of Bird Species by Their Sounds. Master's Thesis. Chapter 2. Helsinki University of Technology.

Fitzpatrick, J., Bates, J., Bostwick, K., Caballero, I., Clock, B., Farnsworth, A. et al. (2004) Family Tyrannidae (Tyrant-flycatchers). pp. 170-462 in J. del Hoyo, A. Elliott and J. Sargatal, eds. Handbook of the Birds of the World, Vol. 9. Lynx Edicions, Barcelona.

Freckleton, R. P. & Jetz, W. (2009) Space versus phylogeny: disentangling phylogenetic and spatial signals in comparative data. *Proceedings of the Royal Society B*, **276**, 21-30.

Gómez, J. P., Bravo, G. A., Brumfield, R. T., Tello, J. G., & Cadena, C. D. (2010) A phylogenetic approach to disentangling the role of competition and habitat filtering in community assembly of Neotropical forest birds. *Journal of Animal Ecology,* **79**, 1181-1192.

Hawkins, B. A., Diniz-Filho, J. A. F., Bini, L. M., Araújo, M. B., Field, R., Hortal, J., Kerr, J. T., Rahbek, C., Rodríguez, M. A. &Sanders, N. J. (2007) Metabolic theory and diversity gradients: where do you go from here? *Ecology*, **88**, 1898-1902.

Hawkins, B. A., Diniz-Filho, J. A. F., Jaramillo, C. A. & Soeller, S. A. (2006) Post-eocene climate change, niche conservatism, and latitudinal gradient of New World birds. *Journal of Biogeography*,**33**,770-780.

Hunter, M. L. & Krebs, J. R. (1979) Geographical variation in the song of great tit (*Parus major*), in relation to ecological factors. *Journal of Animal Ecology*, **48**, 759-785.

Isler, M.L., Isler, P. R. & Whitney, B. M. (2005) Clinal variation in vocalizations of an antbird (Thamnophilidae) and implications for defining species limits. Auk 122: 433-444.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D. D. Blomberg, S. P. & Webb. C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463-1464.

Kerkhoff, A. J., Moriarty, P. E. & Weiser, M. D. (2014) The latitudinal species richness gradient in New World woody angiosperms is consistent with the tropical conservatism hypothesis. *Proceedings of National Academic Science*, **111**, 8125-8130.

Kirschel, A. N. G, Blumstein, D. T., Cohen, R. C., Buermann, W., Smith,T. B. & Slabbekoorn, H. (2009) Birdsong tuned to the environment: green hylia song varies with elevation, tree cover, and noise. *Behavioral Ecology*, **20**, 1089-1095.

Klicka, J., Voelker, G. & Spellman, G. M. (2005) A molecular phylogenetic analysis of the "true thrushes" (Aves: Turdinae). *Molecular Phylogenetics and Evolution,* **34**, 486-500.

Kroodsma, D. E. (1977) Correlates of song organization among North American wrens. *American Naturalist*, **111**, 995-1008.

Leonard, M. L. & Horn, A. G. (2001) Begging calls and parental feeding decisions in tree swallows (*Tachycineta bicolor*). *Behavioral Ecology and Sociobiology*, **49**, 170-175.

Kroodsma, D. E. & Konishi, M. (1991) A suboscine bird (eastern phoebe, *Sayornis phoebe*) develops normal song without auditory feedback. *Animal Behaviour*, **42**, 477-487.

Kroodsma, D.E. & Miller, E. H. (1996) *Ecology and Evolution of Acoustic Communication in Birds.* Cornell University Press, Ithaca, NY, 1996.

Lemon, R. E. (1967) The response of cardinals to songs of different dialects. *Animal Behaviour*, **15**, 538-545.

Lemon, R. E., Struger, J., Lechowicz, M. J. & Norman, R. F. (1981) Song features and singing heights of American warblers: maximization or optimization of distance? *Journal of the Acoustical Society of America*, **69**, 1169-1176.

Liu, I. A., Lohr, B., Olsen, B. & Greenberd, R. (2008) Macrogeographic vocal variation in subspecies of swamp sparrow. *Condor*, **110**, 102-109.

Lovette, I. J., Pérez-Emán, J. L., Sullivan, J. L., Banks, R. C., Fiorentino, I., Córdoba-Córdoba, S. *et.al*. (2010) A comprehensive multilocus phylogeny for the Wood-Warblers and a revised classification of the Parulidae (Aves). *Molecular Phylogeneics and Evolution*, **57**, 753-770.

Marler, P & Slabbekoorn, H. (2004) Nature´s Music. The Science of Birdsong. Hardcover, 504 pages, including 2 CD´s. Academic Press Elsevier, San Diego.

Marques, P. A., Vicente, M. L. & Márquez, R. (2009) Nestling Begging Call Structure and Bout Variation Honestly Signal Need but Not Condition in Spanish Sparrows. *Zoological Studies*, **48**, 587-595.

Mason, N (2012) Song complexity and its evolutionary correlates across a continent-wide radiation of songbirds. MSc thesis, San Diego State University..

Morton, E. S. (1975) Ecological sources of selection on avian sounds. *American Naturalist*, **109**, 17-34.

Nelson, D. A. (1992) Song overproduction and selective attrition lead to song sharing in the field sparrow. *Behavioral Ecology and Sociobiology*, **30**, 415-424.

Nicholls, J. A. & Goldizen, A. W. (2006) Habitat type and density influence vocal signal design in satin bowerbirds. *Journal of Animal Ecology*, **75**, 549-558.

Nowicki, S. & Searcy, W. (2004) Song function and the evolution of female preferences: Why birds sing and why brains matter. Behavioral Neurobiology of Birdsong (eds. H.P. Zeigler & P. Marler), pp. 1-37. New York Academy of Sciences Press.

Nulty, B., Burt, J. M., Akçay, Ç., Templeton, C., Campbell, S. E. & Beecher, M. D. (2010) Song learning in song sparrows: relative importance of autumn vs. spring tutoring. *Ethology*, **116**, 653-661.

Ohlson, J. I., Fjeldså, J., Per, G. & Ericson, P. (2013) Molecular phylogeny of the manakins (Aves: Passeriformes: Pipridae), with a new classification and the description of a new genus. *Molecular Phylogenetics and Evolution*, **69**, 796-804.

Ohlson, J., Fjeldså, J. & Ericson, P. G. P. (2008) Tyrant flycatchers coming out in the open: phylogeny and ecological radiation of Tyrannidae (Aves, Passeriformes). *Zoologica Scripta,* **37**, 315-335.

Olalla-Tárraga, M. A., Diniz-Filho, J. A. F., Bastos, R. P. & Rodríguez, M. A. (2009) Geographic body size gradients in tropical regions: water deficit and anuran body size in the Brazilian Cerrado. *Ecography*, **32**, 581-590.

Podos, J. &Warren, P. S. (2007) The evolution of geographic variation in birdsong. *Advances in the Study of Behavior*, **37**, 403-458.

Pinheiro, J., D. Bates, DebRoy, S., Sarkar, D & R Development Core Team. (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-109.

Price, T. (1998) Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society B*, **353**, 251-260.

R Development Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Ramirez, L., Diniz-Filho, J. A. F. & Hawkins, B. A. (2008) Partitioning phylogenetic and adaptive components of the geographical body-size pattern of New World birds. *Global Ecology and Biogeography*, **17**, 100-110.

Rangel, T. F. L. B., Diniz-Filho, J. A. F. & Cowell, R. K. (2007) Species richness and evolutionary niche dynamics: a spatial pattern-oriented simulation experiment. *American Naturalist*, **170**, 1-15.

Richards, D. G. & Wiley, R. H. (1980) Reverberations and amplitude fluctuations in the propagation of sound in a forest: implications for Animal communication. *American Naturalist*, **115**, 381-399.

Ryan, M. J. & Brenowitz, E. A. (1985) The role of Body Size, Phylogeny and Ambient Noise in the evolution of Bird Song. *American Naturalist*, **126**, 87-100.

Seddon, N. (2005) Ecological adaptation and species recognition drives vocal evolution in Neotropical suboscine birds. *Evolution*, **59**, 200-215.

Seddon, N. & Tobias, J. A. (2007) Song divergence at the edge of Amazonia: an empirical test of the peripatric speciation model. *Biological Journal of the Linnean Society*, **90**, 173-188.

Slabbekoorn, H. & Smith, T. B. (2002) Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society B*, **357**, 493-503.

Slabbekoorn, H., Ellers, J. & Smith, T. B. (2002) Birdsong and sound reverberation: the benefits of reverberations. *Condor*, **104**, 564.

Slater, P. J. B. (1989) Bird song learning: causes and consequences. *Ethology Ecology and Evolution*, **1**, 19-46.

Snow, D. (2004) Family Pipridae (Manakins). pp. 110-169 in J. del Hoyo, A. Elliott and J. Sargatal, eds. Handbook of the Birds of the World, Vol. 9. Lynx Edicions, Barcelona.

Specht, R. (2011) Avisoft SAS Lab Pro Sound Analysis and Synthesis Laboratory. Version 5.1. Avisoft, Berlin, Germany.

Suthers, R. A. & Hector, D. H. (1982) Mechanism for the Production of Echolocation Clicks by the Grey Swiftlet, *Collocmia spodiopygia*. *Journal of Comparative Physiology*, **148**, 457-470.

Suthers, R. A., & Hector, D. H. (1985) The physiology of vocalization by the echolocating oilbird, *Steatornis caripensis. Journal of Comparative Physiology*, **56**, 243-266.

Suthers, R. A. & Zollinger, S. A. (2004) Producing song: the vocal apparatus. *Annals of the* New York *Academy of Sciences*, **1016**, 109-129.

Tobias, J. A., Aben, J., Brumfield, R. T., Derryberry, E. P., Halfwerk, W., Slabbekoorn, H. & Seddon, N. (2010) Song Divergence by Sensory Drive in Amazonian Birds. *Evolution, **64***, 1-20.

Tubaro, P. L. & Mahler, B. (1998) Acoustic frequencies and body mass in New World doves. *Condor*, **100**, 54-61.

Tubaro, P. L & Segura, E.T. (1994) Song of *Zonotrichia capensis* in the southern pampas: a test of the acoustic adaptation hypothesis. *Condor*, **96**, 1084-1088.

Vinciková, H., Hais, M., Brom, J., Procházka, J., Pecharová, E. (2010) Use of remote sensing methods in studying agricultural landscapes – a review. *Journal of Landscape Studies*, 3, 53-63.

Wallschlager. D. (1980) Correlation of song frequency and body weight in passerine birds. *Experientia* 36: 412.

Weir, J. T., Wheatcroft, D. & Price. T. (2012) The role of ecological constraint in driving the evolution of avian song frequency across a latitudinal gradient. *Evolution,* **66**, 2773-2783.

Weir, J. T. & Wheatcroft, D. (2011) A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Proceedings of Royal Society London B*, **278**, 1713-1720.

Wiley, R. H. & Richards, D. G. (1982) Adaptations for acoustic communication in birds: sound transmission and signal detection. *Acoustic Communication in Birds* (eds D. E. Kroodsma & E. H. Miller), pp. 131-181.Academic Press, New York and London.

Wiley, R. H. (1991) Associations of song properties with habitats for territorial oscine birds of eastern North America. *American Naturalist*, **138**, 973-993.

Wiens, J. J., Graham, C. H., Moen, D., Smith,S. A. & Reeder, T. W. (2006) Evolutionary and ecological causes of the latitudinal diversity gradient in Hylid frogs: treefrog trees unearth the roots of high tropical diversity. *American Naturalist*, **168**, 579-596.

Zimmer, K. J., &Isler, M. L. (2003) Family Thamnophilidae (typical antbirds). pp. 448–68 in J. del Hoyo, A. Elliott, and D. Christie, eds. Handbook of birds of the World. Vol. 8. Lynx Edicions, Barcelona.

FI GURE CAPTIONS

Figure 1. Thamnophilidae song variables affected by Normalized Difference Vegetation Index (NDVI). (a): Residuals of peak frequency vs. richness; (b): Residuals of maximum frequency vs. richness; (c): Residuals of minimum frequency vs. richness; (d): NDVI based on average of values calculated per species present in each cell using the interpolation method (see Methods). All these frequency variables showed an inverse relation with NDVI, according with specific predictions of AAH of lower frequency values associated to closed vegetation (or high NDVI values).

Figure 2. Tyrannidae song variable affected by Normalized Difference Vegetation Index (NDVI). (a) Residuals of Maximum frequency vs. richness; (b): NDVI based on average of values calculated per species present in each cell using the interpolation method (see Methods). The lowest residual values to maximum frequency (a) were associated to highest NDVI values (b), confirming the AAH predictions related to frequency song parameters.

Figure 3. Pipridae song variables affected by Normalized Difference Vegetation Index (NDVI). (a): Residuals of peak frequency vs. richness; (b): Residuals of maximum frequency vs. richness; (c): Residuals of frequency bandwidth vs. richness; (d): NDVI based on average of values calculated per species present in each cell using the interpolation method (see Methods). Contrasting with AAH predictions there is a positive relation between frequency variables and NDVI, with lower frequency values associated to more open vegetation (lower NDVI values).

Figure 4. Turdidae song variables affected by Normalized Difference Vegetation Index (NDVI). (a): Residuals of peak frequency vs richness; (b): Residuals of minimum frequency vs richness; (c):NDVI based on average of values calculated per species present in each cell using the interpolation method (see Methods). As in Pipridae songs, lower

frequency values were positively associated to lower NDVI values, indicating the opposite pattern that expected by AAH.

Figure 1.

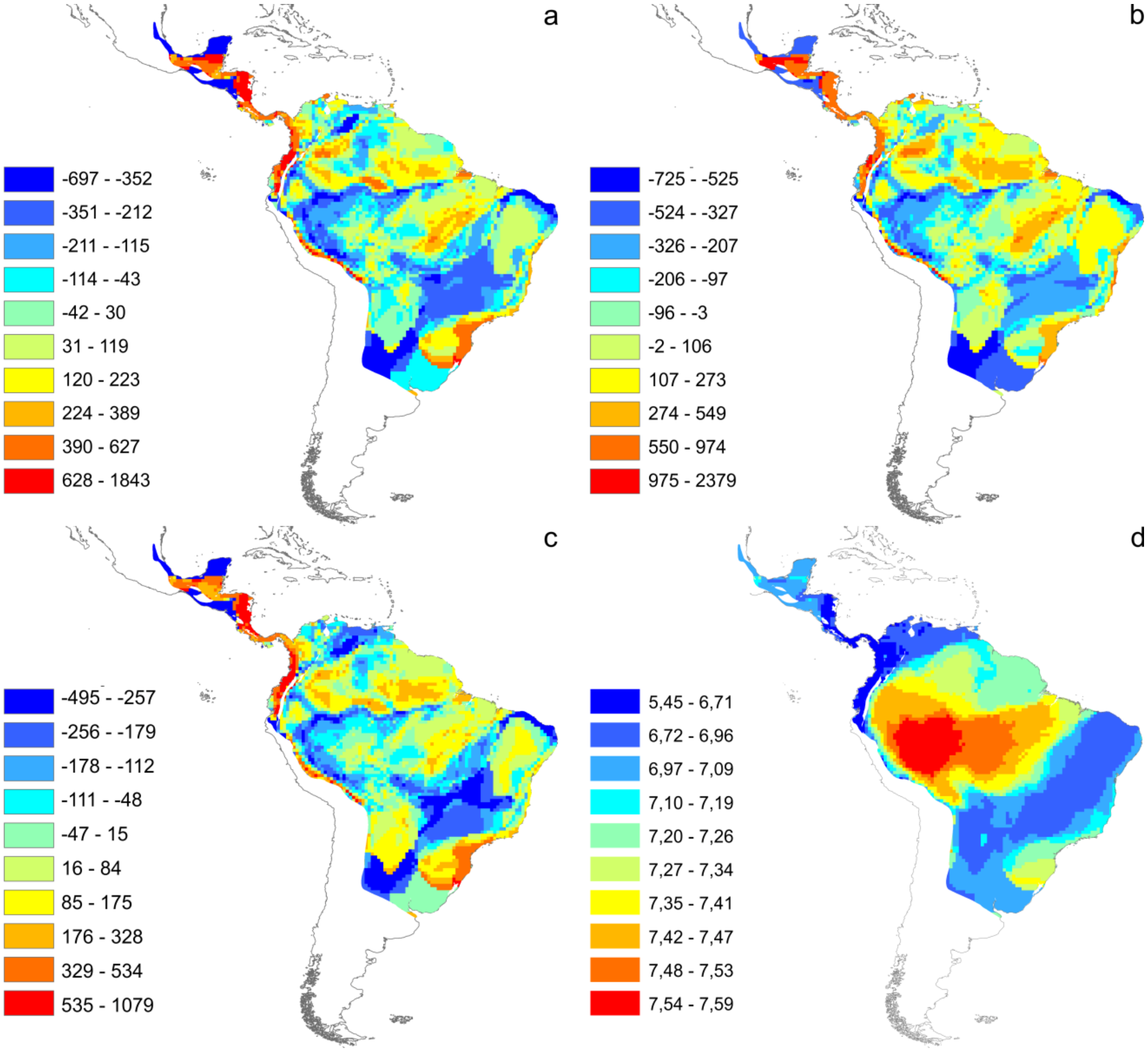
a
-697 - -352
-351 - -212
-211 - -115
-114 - -43
-42 - 30
31 - 119
120 - 223
224 - 389
390 - 627
628 - 1843
b
-725 - -525
-524 - -327
-326 - -207
-206 - -97
-96 - -3
-2 - 106
107 - 273
274 - 549
550 - 974
975 - 2379
c
-495 - -257
-256 - -179
-178 - -112
-111 - -48
-47 - 15
16 - 84
85 - 175
176 - 328
329 - 534
535 - 1079
d
5,45 - 6,71
6,72 - 6,96
6,97 - 7,09
7,10 - 7,19
7,20 - 7,26
7,27 - 7,34
7,35 - 7,41
7,42 - 7,47
7,48 - 7,53
7,54 - 7,59

Figure 2.

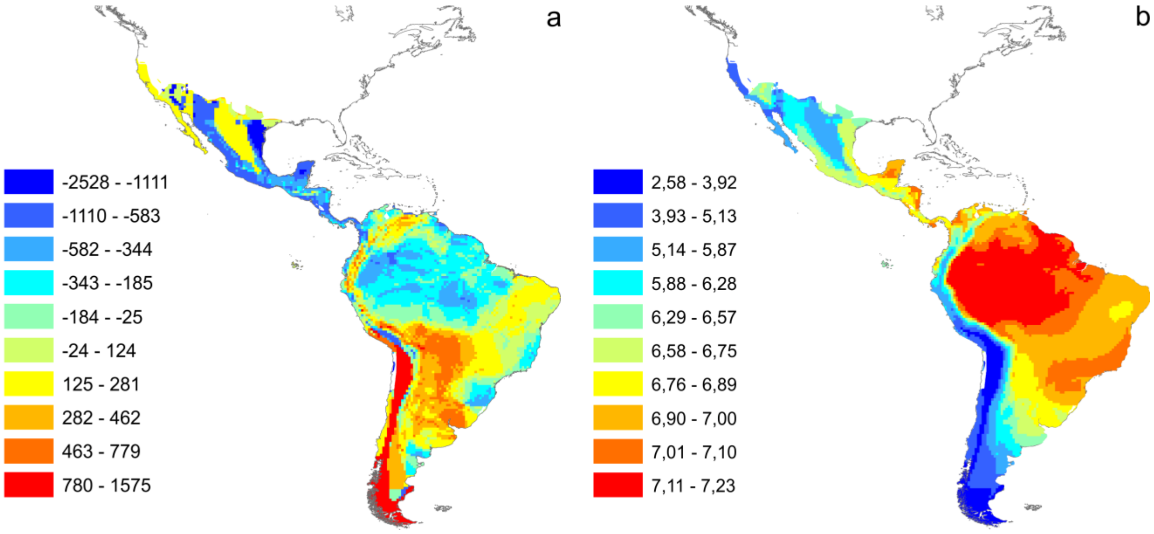
a
-2528 - -1111
-1110 - -583
-582 - -344
-343 - -185
-184 - -25
-24 - 124
125 - 281
282 - 462
463 - 779
780 - 1575
b
2,58 - 3,92
3,93 - 5,13
5,14 - 5,87
5,88 - 6,28
6,29 - 6,57
6,58 - 6,75
6,76 - 6,89
6,90 - 7,00
7,01 - 7,10
7,11 - 7,23

Figure 3

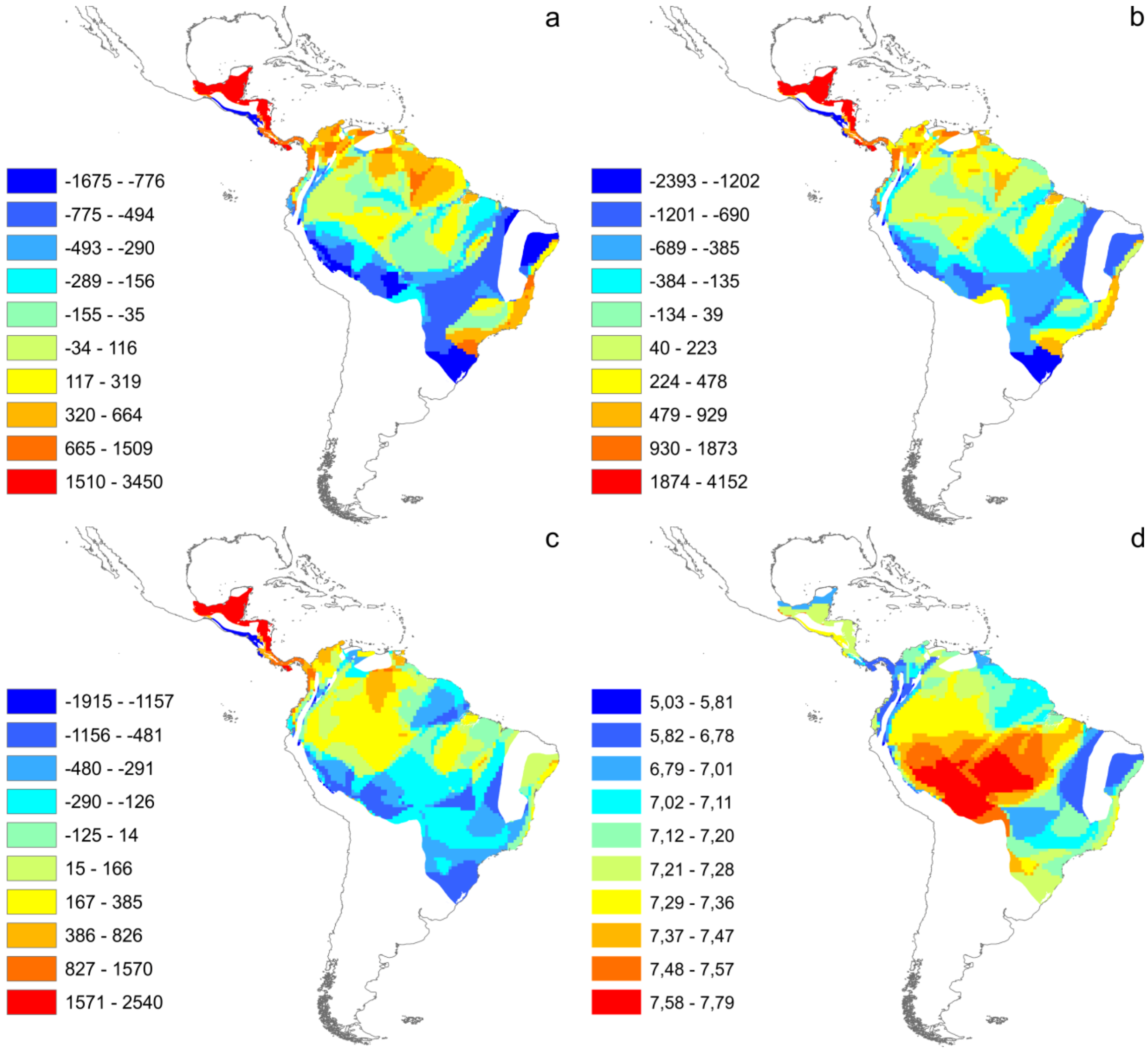
a
-1675 - -776
-775 - -494
-493 - -290
-289 - -156
-155 - -35
-34 - 116
117 - 319
320 - 664
665 - 1509
1510 - 3450
b
-2393 - -1202
-1201 - -690
-689 - -385
-384 - -135
-134 - 39
40 - 223
224 - 478
479 - 929
930 - 1873
1874 - 4152
c
-1915 - -1157
-1156 - -481
-480 - -291
-290 - -126
-125 - 14
15 - 166
167 - 385
386 - 826
827 - 1570
1571 - 2540
d
5,03 - 5,81
5,82 - 6,78
6,79 - 7,01
7,02 - 7,11
7,12 - 7,20
7,21 - 7,28
7,29 - 7,36
7,37 - 7,47
7,48 - 7,57
7,58 - 7,79

Figure 4.

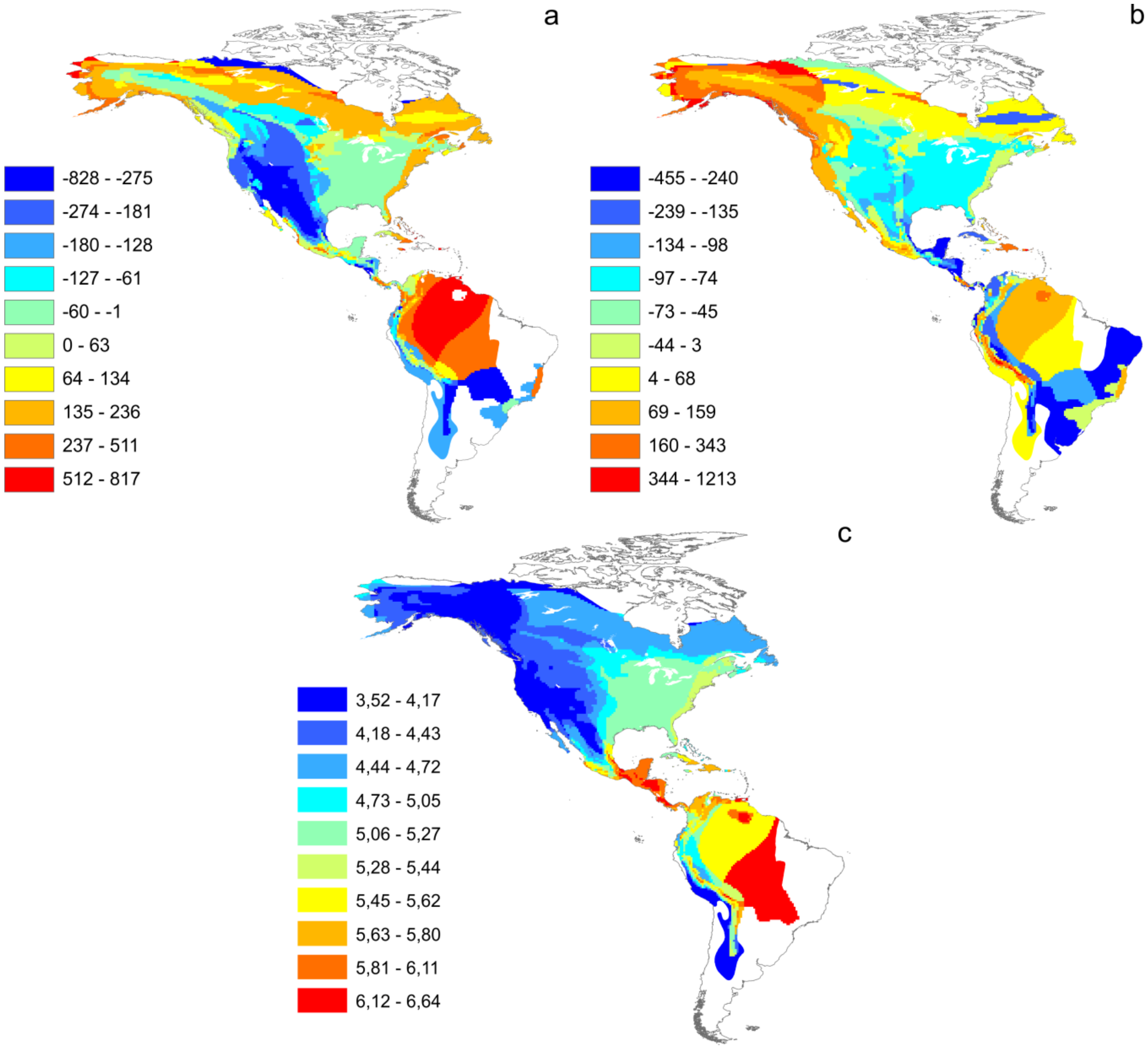
a
-828 - -275
-274 - -181
-180 - -128
-127 - -61
-60 - -1
0 - 63
64 - 134
135 - 236
237 - 511
512 - 817
b
-455 - -240
-239 - -135
-134 - -98
-97 - -74
-73 - -45
-44 - 3
4 - 68
69 - 159
160 - 343
344 - 1213
c
3,52 - 4,17
4,18 - 4,43
4,44 - 4,72
4,73 - 5,05
5,06 - 5,27
5,28 - 5,44
5,45 - 5,62
5,63 - 5,80
5,81 - 6,11
6,12 - 6,64

Table 1.Number of species and recordings per family with mean and standard deviation of each song trait analysed in this study.

	Thamnophilidae	Tyrannidae	Pipridae	Parulidae	Turdidae
Number of species	122	77	35	75	27
Number of recordings	842	684	220	414	1013
Mean number of recordings per species (range)	6.902 (1-31)	8.883 (1-40)	6.286 (1-22)	15.333 (1-30)	13.507 (1-27)
Song duration (SD) (s)	3.05 ± 2.88	1.18 ± 0.86	0.71 ± 0.57	2.22 ± 1.30	2.66 ± 2.69
Number of notes (NN)	15.03 ± 10.07	9.96 ± 10.04	3.56 ± 2.96	15.63 ± 10.50	8.92 ± 12.86
Element rate (ER)	0.27 ± 0.19	0.16 ± 0.16	0.21 ± 0.34	0.17 ± 0.05	0.28 ± 0.16
Peak frequency (PF) (Hz)	2988.99 ± 1300.35	4223.66 ± 1436.26	3694.72 ± 1565.18	5262.17 ± 1104.91	3360.54 ± 853.66
Maximum frequency (Fmax) (Hz)	3647.70 ± 1576.18	5293.19 ± 1800.11	4670.72 ± 1755.39	7481.44 ± 1451.69	5512.43 ± 1721.71
Minimum frequency (Fmin) (Hz)	2002.69 ± 1009.00	2225.09 ± 1178.88	2141.18 ± 1231.83	3114.84 ± 1024.71	2020.70 ± 556.23
Frequency bandwidth (FB) (Hz)	1645.01 ± 827.61	3068.11 ± 1413.95	2529.54 ± 1138.22	4366.60 ± 1162.09	3491.74 ± 1673.46
Number of different note types (NDN)	1.90 ± 0.61	2.25 ± 0.97	1.74 ± 0.82	4.75 ± 3.78	4.93 ± 4.51

Table 2. Proportional contributions of spatial (φ), phylogenetic (λ'), and independent (γ) effects on variation in song characteristics of different bird families. Thamnophilidae and Parulidae

Family	Variable	Log likelihood	γ	λ'	Φ
Thamnophilidae (N=122 species)	Duration	-250.981	0.01	0.98*	0.01
	number of notes	-447.778	0.54	0.45	0.01
	element rate	33.742	0.25	0.74*	0.01
	peak frequency	-972.145	0.02	0.91*	0.06
	maximum frequency	-998.011	0.03	0.96*	0.01
	minimum frequency	-961.196	0.01	0.98*	0.01
	frequency bandwidth	-959.18	0.03	0.8*	0.17
Tyrannidae (N=77 species)	Duration	-155.709	0.01	0	0.99
	number of notes	-283.62	0.51	0.01	0.49
	element rate	-605.874	0.01	0	0.99
	peak frequency	-666.338	0.54	0.45	0.01
	maximum frequency	-679.89	0.83	0.16	0.01
	minimum frequency	-656.893	0.04	0.23	0.73
	frequency bandwidth	-662.54	0.78	0.01	0.21
Pipridae (N= 35 species)	Duration	-21.057	0.98	0.01	0.01
	number of notes	-85.787	0.98	0.01	0.01
	element rate	-2.243	0.01	0	0.99
	peak frequency	-298.55	0.61	0.38	0.01
	maximum frequency	-303.944	0.64	0.35	0.01
	minimum frequency	-286.359	0.2	0.79	0.01
	frequency bandwidth	-288.673	0.91	0.01	0.08
Parulidae (N=75 species)	Duration	-100.58	0.01	0	0.99
	number of notes	-271.188	0.01	0.65*	0.34
	element rate	114.773	0.01	0.71*	0.28
	peak frequency	-622.493	0.76	0.01	0.23

	maximum frequency	-640.227	0.06	0.93*	0.01
	minimum frequency	-620.288	0.7	0.01	0.29
	frequency bandwidth	-630.143	0.39	0.6*	0.01
Turdidae (N=27 species)	Duration	-95.09	0.01	0.98	0.01
	number of notes	-95.09	0.01	0.98	0.01
	element rate	14.37	0.63	0.01	0.36
	peak frequency	-211.12	0.98	0.01	0.01
	maximum frequency	-237	0.98	0.01	0.01
	minimum frequency	-198.69	0.98	0.01	0.01
	frequency bandwidth	-237.52	0.98	0.01	0.01

Table 3. Results from generalized least squares analyses on the relationship between song acoustic properties and the average Normalized Difference Vegetation Index (NDVI) of the corresponding species for different bird families. NDVI* mass are the models in which log of body mass by species was included as interaction with NDVI. PF: Peak frequency (KHz); Fmax: maximum frequency (KHz); Fmin: minimum frequency (KHz); FB: frequency bandwidth (KHz); SD: song duration (s); NN: number of notes (or elements); ER: element rate (mean duration of the element). Significant results are evidenced in bold.

Family		PF (KHz)		Fmax (KHz)		Fmin (KHz)		FB (KHz)		SD (s)		NN		ER	
		NDVI	NDVI *mass	NDVI	NDVI *mass	NDVI	NDVI *mass	NDVI	NDVI *mass	NDVI	NDVI *mass	NDVI	NDVI *mass	NDVI	NDVI *mass
Thamnophilidae	Slope	-201.2	169.31	-195.78	46.13	-115.85	83.74	-76.58	-100.4	0.2443	0.2827	0.205	-0.864	0.01309	0.13272
	SE	73.66	124.04	84.62	127.15	52.98	61.56	73.24	152.42	0.1569	0.1824	1.307	5.862	0.02399	0.08376
	t	-2.731	1.365	-2.314	0.363	-2.187	1.36	-1.046	-0.659	1.556	1.55	0.157	-0.147	0.546	1.585
	p	**0.007**	0.17487	**0.0224**	0.7174	**0.0308**	0.1763	0.298	0.511	0.122	0.124	0.875	0.883	0.5863	0.1158
Tyrannidae	Slope	-251.31	194.07	-396.9	117.6	-47.75	40.49	-268.195	0.8223	0.0802	0.1274	-0.763	0.384	-17.75	36.2
	SE	145.85	130.7	175	127.8	126.9	70.85	140.466	121.879	0.1931	0.0955	1.047	1.036	66.81	33.04
	t	-1.723	1.485	-2.268	0.92	-0.376	0.571	-1.909	0.007	0.415	1.335	-0.729	0.372	-0.266	1.096
	p	0.089	0.14194	**0.0263**	0.3607	0.7078	0.56946	0.0602	0.9946	0.679	0.186	0.468	0.711	0.791	0.277
Pipridae	Slope	195.1	3466.9	314.6	3467.6	277.5	1144	173.8	1677.5	-0.145	0.507	-0.145	0.507	0.0713	0.24344
	SE	319.7	1083	374.4	1264.1	207.3	647.8	252.1	787.5	0.7662	2.4281	0.766	2.428	0.06063	0.14066
	T	0.61	3.201	0.84	2.743	1.339	1.766	0.689	2.13	-0.189	0.209	-0.189	0.209	1.176	1.731

	p	0.546	**0.00323**	0.4074	**0.0102**	0.1907	0.0876	0.49584	**0.04149**	0.851	0.836	0.851	0.836	0.24882	0.09378
Parulidae	Estimate	2.147	-607.376	116.51	-6.298	-45.63	-226.43	-18.54	-556.57	-0.1074	-0.1808	-0.325	-1.476	0.000261	0.007336
	SE	115.399	313.879	133.426	188.509	112.87	307.91	127.74	324.69	0.1103	0.2117	0.995	1.628	0.005716	0.008964
	t	0.019	-1.935	0.873	-0.033	-0.404	-0.735	-0.145	-1.714	-0.974	-0.854	-0.327	-0.906	0.046	0.818
	p	0.985	0.057	0.386	0.973	0.687	0.465	0.885	0.0909	0.3334	0.396	0.744	0.368	0.964	0.416
Turdidae	Slope	133.6	794.8	218.7	660.4	23.18	628.22	195.49	32.22	-1.46	-3.379	-1.46	-3.379	-0.007988	-0.101368
	SE	106.4	227.6	277.5	593.7	67.15	143.67	282.94	605.38	1.424	2.865	1.424	2.865	0.02671	0.06879
	t	1.256	3.491	0.788	1.112	0.345	4.373	0.691	0.053	-1.025	-1.18	-1.025	-1.18	-0.299	-1.474
	p	0.222	**0.00207**	0.439	0.278	0.73324	**0.00024**	0.497	0.958	0.316	0.251	0.316	0.251	0.768	0.155

4. ARTIGO II

Testing alternative models of songbird evolution

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Testing alternative models of songbird evolution

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5.1 Abstract

Acoustic communication has been a hallmark of avian evolution, yet little is known about the mode and tempo of evolution of the birdsongs. In this study we fit four alternative models of evolution (White Noise, Brownian motion, Ornstein-Uhlenbeck and Early-Burst) on song characteristics of suboscine (Thamnophilidae, Tyrannidae and Pipridae) and oscine families (Parulidae and Turdidae). Interspecific variation in acoustic traits was summarized using principal component analyses and the resulting scores were used for model fitting. The first PC, which accounted for approximately 40% of the variance in all datasets, indicated that the main axis of birdsong evolution involves changes in frequency parameters. In addition, the simplest models (WN and BM) showed the best fit to most of song principal component scores. Despite extensive evidence from the literature on the role of environment and morphology in constraining song frequency, these constraints do not seem to translate into macroevolutionary timescales. One possibility for this discrepancy is that birdsong plasticity might ensue due to its multivariate nature: song is the result of temporal and frequency components and birds can change some of these components depending on their singing context.

Keywords: Oscines, Suboscines, Brownian motion, Ornstein-Uhlenbeck, phylogenetic comparative methods, Early Burst, acoustic communication.

5.2. Background

Birds are among the animals with the highest diversity in acoustic communication. There is considerable variation in the types of signals produced by birds, including songs, mechanical sounds, alarm calls, and echolocation (Suthers & Hector 1982, 1985, Prum 1998, Catchpole & Slater 2008). Among all these vocalization types, songs have been the main focus of bioacoustic research, given that they play important roles in the contexts of sexual attraction and territorial defence and therefore are likely to be under strong natural and sexual selection (Slater 1989, Price 1998, Nowicki & Searcy 2004). Historically, phylogenetic comparative methods have been used in birdsong studies to reconstruct vocal evolution in some clades (Price & Lanyon 2002, 2004, Price *et al*. 2007), to test the correlated evolution between song and morphology or environmental conditions (Seddon 2005, Tobias & Seddon 2009, Cardoso 2010, Tobias *et al.* 2010), or to assess the level of evolutionary conservatism in certain song characteristics (Marler & Pickert 1984, Payne 1986, Price & Lanyon 2002). However, despite an extensive literature on song evolution, little attention has been given to understand the tempo and mode of evolution of song characteristics.

Interspecific patterns of song diversity suggest that their distribution it is not equal: some clades show considerable differences while others exhibit little variation, suggesting that mechanisms that shape song evolution could act in a different way depending on clade and region. For example, at low-latitude tropical forests, songs seem to have experienced more environmental pressure when compared to open habitats or at higher latitudes, yet song length seems unaffected by latitude in either oscines or suboscines (Weir *et al*. 2012). Furthermore, variation in syllable diversity shows a gradient in oscine songs, which exhibit rates 20 times higher at temperate latitudes (Weir & Wheatcroft 2011). These examples show that song diversification is not constant, but instead can be accelerated or decelerated according to context where bird lineages evolve.

Recent advances in phylogenetic comparative methods allow for fitting more realistic models of song trait evolution than the classical Brownian motion (BM) or Ornstein-Uhlenbeck (OU) models, the two more commonly used models in phylogenetic comparative analysis (Felsenstein 1985, Butler & King 2004). Brownian motion is a neutral model, in which the variance in a given trait accumulates at a constant rate along each branch of the phylogeny. In this model the differences between species traits grow linearly with the evolutionary time, and there is not an adaptive component (Dias-Uriarte and Garland Jr. 1996, Pagel 1997, Blomberg *et al.* 2003, Freckleton and Jetz 2009). On the contrary, in the OU model the phenotypic traits are subject to genetic or environmental constraints, being constraint defined as the property of a trait that, although possibly adaptative in the environment in which it originally evolved, acts to place limits on the production of new phenotypics variants (Butler and King 2004). More recently Blomberg *et al.* 2003 and Harmon *et al.* (2010), developed the Early Burst model (or ACDC model), in which the earliest divergences in the phylogenetic tree account for a substantial fraction of the total trait disparity in a clade (Harmon. We argue that the application of alternative models on song traits can provide valuable insights into the mechanisms underlying birdsong evolution.

In this study we explored the fit of four models of evolution: White Noise (non phylogenetic model), Brownian motion, Ornstein-Uhlenbeck and Early-Burst on song parameters of suboscine (Thamnophilidae, Tyrannidae and Pipridae) and oscine families (Turdidae and Parulidae). We used the models as exploratory tools for comparing patterns of song evolution between families in which the song is either innate (suborder Suboscines) or learned (suborder Oscines). We believe that testing the relative fit of evolutionary models using families with a broad variation in ecology, behaviour, morphology and distribution can give us insights about differences in the evolutionary processes shaping birds songs.

5.3. Methods

We measured a total of 3173 songs from three suboscine (Thamnophilidae, Tyrannidae and Pipridae) and two oscine families (Turdidae and Parulidae) (Table 1).

Recordings were obtained from the following collections: Instituto de Investigación de Recursos Biológicos Alexander Von Humboldt (Colombia), Acervo Neotropical Elias Coelho (Universidade Federal do Rio de Janeiro, Brazil), Macaulay Library (Cornell University, EUA), XENO-CANTO database (http://www.xeno-canto.org), and private song collections of Jeremy Minns and Christian Borges Andretti. Songs with sample rate and resolution less than 22.050 Hz and 16 bits were discarded. For suboscines we measured one phrase in each song per individual, given this suborder shows stereotyped songs with little variation in repertoire. However, in oscines, due to learning, one individual can sing a large number of different phrases within a song. In this case, to capture the song variation throughout a species, we analysed all the different phrases sung by each individual within an interval of three minutes.

Spectrograms were generated using the software AVISOFT SAS Lab Pro 5.1, with the following specifications: Window: Hamming, FFT: 256, Frame Size: 100%, and Overlap: 88%. We used the “two thresholds” automatic parameters with the threshold fitted to each song independently, visualizing the best cutoff value in the power spectrum graphic, allowing the selection of all notes and excluding the noise in the background. For all songs, we estimated the following acoustic measurements: 1) mean song duration (SD): (s), the duration from the beginning of the first element (note) to the end of the last element in the song; 2) mean number of notes (NN): number of elements (notes) detected within the song; 3) element rate (ER): (s), the average duration of the elements (notes) within a song;4) peak frequency (PF): (KHz), at maximum spectrum (peak hold) of the entire song; 5) Maximum frequency (F_{max}): (kHz), the highest frequency across the entire song; 6) Minimum frequency (F_{min}): (kHz), the lowest frequency across the entire song; 7) Frequency Bandwidth (FB): (KHz), the range in frequency values within a song. FB was obtained by subtracting

F_{min} from F_{max}; 8) Number of different notes (NDN): the number of different note types found within a phrase. NDN was used as a proxy for song complexity.

To measure the F_{min} and F_{max} we used the manual cursor, given songs showed considerable variation in relation to the presence of harmonics and, as a consequence, the accurate automatic estimation of these parameters could be compromised. Figure 1 illustrates these quantitative measures in a spectrogram. To each family, we reduced the dimensionality in the song dataset through a Principal Component Analysis (PCA), using correlation matrix on the standardized mean values within species and the broken stick criterion to select PC axes for further analyses.

We obtained estimates of relative divergence times of the studied lineages using the following phylogenies: Thamnophilidae (Gomez *et al.* 2010), Tyrannidae (Ohlson *et al.* 2008), Pipridae (Ohlson *et al.* 2013), Turdidae (Klicka *et al.* 2005) and Parulidae (Lovette *et al.* 2010). We used the “fitContinuous” function in GEIGER 2.0.1 (Harmon *et al.* 2008) to fit four evolutionary models for each song principal component separately, namely White Noise (WN), Brownian motion (BM), Ornstein-Uhlenbeck (OU) (with one peak) and Early Burst (EB). WN is a non phylogenetic model, which predicts a single normal distribution of the data without a covariance structure among taxa. BM model assumes that the variance in a given trait accumulates at a constant rate along each branch of the phylogeny; OU add the “α” parameter to the BM model, which represent the strength (or constraint) that return the trait to their original state (Butler & King 2004). Finally, the EB model has the “*a*” parameter, which allows the rate of evolution increase or decrease exponentially through time (Harmon *et al.* 2008). The best-fit model for the scores of each principal component was chosen based on the lowest AICc values (Harmon *et al.* 2010).

5.4. Results

Despite substantial differences in ecology, distribution, and behaviour between the studied families, according to their PCAs, much of the structure of the observed variation in song characteristics was largely consistent between them (Table 2). The first PC, which accounted for approximately 40% of the variance in all datasets, indicated that the main axis of birdsong evolution involves changes in frequency parameters (PF, F_{max}, F_{min}, and FB) with fairly similar loadings to Thamnophilidae, Tyrannidae, Parulidae and Pipridae. In Turdidae, however, there was a more homogeneous contribution to PC1 of all variables except frequency bandwidth (Table 2), pointing to a more unique correlation structure for this family. In particular, turdid minimum and maximum frequency loaded most strongly on PCs 2 and 3, respectively. The remaining PCs (PC2-PC4) showed more idiosyncratic patterns for each family, but mostly reflected different aspects of temporal parameters (SD, NN and NR) (Table 2).

A comparison between all four candidate models of evolution (WN, BM, OU, and EB) on each of the song PCs showed that, in general, the simplest models presented the best fit (Table 3). In particular BM and WN models showed the lowest AICc values for the majority of song principal components. In Thamnophilidae, BM was the model with the best fit to both frequency and temporal parameters (PC1 and PC3 respectively). BM was also the preferred model for PC1 and PC4 in Parulidae, and for PC1 in Turdidae. The WN model was the best-fit model to all principal components in Tyrannidae songs, to temporal parameters (SD, NN e SR) and song complexity (NDN) in Pipridae and Turdidae songs (PC2 and PC3 respectively), and to frequency (PC3) in Parulidae songs. The OU and EB models, which represent more complex scenarios, showed similar support according to AICc values as the best fit models to PC2, representing temporal song parameters in Thamnophilidae and temporal and complexity song parameters in Parulidae songs (Table 3).

5.5. Discussion

Contrary to classical ideas suggesting constraints on birdsong imposed by environment, morphology or physiology (Podos *et al*. 2004, Boncoraglio & Saino 2007, Brumm & Naguib 2009, Wilkins *et al*. 2013), our results suggests that, at least in the five families investigated in the present study, acoustic properties of birdsong evolved according to relatively simple rules, with changes in frequency accounting for nearly 40% of the evolution of song acoustic properties. In addition, although there is considerable variation in ecology, morphology and behaviour among the studied birds (del Hoyo *et al.* 2003, 2004, 2005 and 2010), WN and BM showed the best fit to both frequency and temporal parameters in songs (Table 3). These results suggest that changes in these traits could have taken place at a relatively constant rate along the phylogeny in some families, such as Thamnophilidae, Parulidae and Turdidae, with differences accumulating in relative proportion to divergence among species. On the other hand, the best fit of the WN model in Tyrannidae and EB in Pipridae might suggest that changes in frequency might have been more labile in these families.

Frequency was identified as the most important axis of variation in song evolution, given that frequency traits correlated strongly with the first principal component in four out of five studied families (Table 2). There are many studies suggesting that frequency song parameters are strongly subject to environmental constraint and that species adapt their songs according to the habitat in which they have evolved- Acoustic Adaptation Hypothesis - (Morton 1975, Wiley & Richards 1982, Wiley 1991, Badyaev & Leaf 1997, Slabbekoorn & Smith 2002, Boncoraglio & Saino

2007, Derryberry 2009, Kirschel *et al.* 2009, Tobias *et al.* 2010, Wilkins *et al.* 2013). Furthermore, evidences suggest that morphological or physiological constraints could limit the frequency spectrum in which birds are capable to produce sounds (Podos *et al.* 2004). For instance, the production of some frequency spectra depends on the length of vocal tract, which is related to body size (Podos 1997, Podos 2001, Bradbury & Vehremcamp 1998). Therefore, one could expect that the OU model should fit better to song frequency parameters, given that this model could potentially incorporate these environmental or morphological/physiological constraints, yet it was not the best-fit model in PC1 scores of any of the investigated families. We argue that, despite these real constraints imposed on song evolution, birdsong plasticity might ensue due to its multivariate nature: song is the result of temporal and frequency components and birds can change some of these compounds depending on their singing context. For instance, some species can change their perch height to avoid song degradation while singing (Nemeth *et al.* 2001, Barker & Mennill 2009, Barker *et al.* 2009), change the frequency spectrum or amplitude level of their songs at noisy environments (Brumm & Naguib 2009, Hu & Cardoso 2010, Bermúdez-Cuamatzin *et al.* 2010, Halfwerk *et al.* 2011, Schuster *et al.* 2012), or even modify their singing pattern through the alternation in note repetition in response to other signallers (Bermúdez-Cuamatzin *et al.* 2010, Francis *et al.* 2011). This phenomenon can be observed in the case of wood warblers, where the relationship between syllable repetition and song complexity can lead to the evolution of simple, rather than elaborated songs (Cardoso & Hu 2011). Despite the fact that those authors did not specifically fit models of evolution on song traits, their study used other comparative methods and showed that evolution does not always follows the most complex way, same in traits recognized to be subject to direct sexual selection as birdsong.

Our study results disagree with those found by Weir *et al.* (2012), which supported OU model as the best model to explain song evolution in oscine and suboscine birds from tropical and temperate environments. Those authors claimed that frequency was more constrained in tropical than temperate zones, because of the elevated background noise caused by insects and frequency attenuation present in tropical environments. On the other hand, another study that fitted BM and OU models testing AAH found more support for constraint in temporal and structural song features than frequency parameters (Mason 2012). However, it is important to mention that both authors only fitted OU and BM models to song traits, which could limit their explanatory power. The present study is the first to investigate the fit of alternative evolutionary models on song traits for a large dataset of oscine and suboscine lineages. However, a common caveat among these two studies and our results is that behavioural information associated with the recording context was ignored, given that the analysed recordings are from sound collections, in which this information is frequently not available. Future research fitting evolutionary models on song traits could focus in bird assemblages and field experiments, aiming at collecting behavioural data associated to the recording context (e.g. perch height, presence of co-specific, presence of female, etc.).

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5.6. References

Badyaev, A.V. & Leaf, E. S. 1997. Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* Warblers. *Auk* **114**: 40-46.

Barker, N. K. S & Menill, D. J. 2009. Song perch height in Rufous-and-White Wrens: does behaviour enhance effective communication in a tropical forest? *Ethology* **115**: 897-904.

Barker, N. K. S, Dabelsteen, T. & Mennill, D. J. 2009. Degradation of male and female rufous-and-white wren songs in a tropical forest: effects of sex, perch height, and habitat. *Behaviour* **146**: 1093-1122.

Bermúdez-Cuamatzin, E., Ríos-Chelén, A., Gil, D. & Garcia, C. M. 2010. Experimental evidence for real-time song frequency shift in response to urban noise in a passerine bird*. Biol. Lett.***7***:* 36-38.

Blomberg, S. P., Garland Jr., T., Ives, A. R. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **57**: 717-745.

Boncoraglio, G. & Saino, N. 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Funct. Ecol.* **21**: 134-142.

Bradbury, J. W. & Vehrencamp, S. L. 1998. Principles of animal communication. Sinauer, Sunderland, MA.

Butler, M. A. & King, A. A. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* **164**: 683-695.

Brumm, H. & Naguib, M. 2009. Environmental Acoustics and the Evolution of Bird Song. *Adv. Stud. Behav.* **40**: 1-33.

Cardoso, G. C. & Hu, Y. 2011. Birdsong Performance and the Evolution of Simple (Rather than Elaborate) Sexual Signals. *Am. Nat.* **178**: 679-686.

Catchpole, C. K. & Slater, P. J. B. 2008. Bird song: Biological Themes and Variations, 2nd ed. Cambridge, UK: Cambridge University Press.

Collar, N. 2005. Family Turdidae (Thrushes). *In*: Handbook of the Birds of the World, Vol. 10. Cuckoo-shrikes to Thrushes (J.del Hoyo, A. Elliott & J. Sargatal), pp. 514-807. Lynx Edicions, Barcelona.

Curson, J. 2010. Family Parulidae (New World Warblers). *In*: Handbook of the Birds of the World, Vol.15. Weaves to New World Warblers (J. del Hoyo, A. Elliott & J. Sargatal), pp. 666-800. Lynx Edicions, Barcelona.

Derryberry, E. 2009. Ecology Shapes Birdsong Evolution: Variation in Morphology and Habitat Explains Variation in White-Crowned Sparrow Song. *Am. Nat.* **174**: 24-33.

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1-15.

Fitzpatrick, J., Bates, J., Bostwick, K., Caballero, I., Clock, B., Farnsworth, A. et al 2004. Family Tyrannidae (Tyrant-flycatchers). Handbook of the Birds of the World, Vol. 9. Cotingas to Pipits and Wagtails (J.del Hoyo, A. Elliott & J. Sargatal), pp. 170-462. Lynx Edicions, Barcelona.

Francis, C. D., Ortega, C. P. & Cruz. A. 2011. Different behavioural responses to anthropogenic noise by two closely related passerine birds. *Biol. Lett*. **7**: 850-852.

Gómez, J. P., Bravo, G. A., Brumfield, R. T., Tello, J. G., & Cadena, C. D. 2010. A phylogenetic approach to disentangling the role of competition and habitat filtering in community assembly of Neotropical forest birds. *J. Anim. Ecol.* **79**: 1181-1192.

Halfwerk, W., Bot, S., Buikx, J., van der Velde, M., ten Cate, C. & Slabbekoorn, H. 2011. Low-frequency songs lose their potency in noisy urban conditions. *PNAS* 108: 14549-14554.

Harmon, L. J., Losos, J. B., Davies, T. J., Gillespie, R. G., Gittleman, J. L., Jennings, B. et al. 2010. Early bursts of body size and shape evolution are rare in comparative data. *Evolution* **64**: 2385-2396.

Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**: 129-131.

Harmon, L. J., Schulte, J. A., Losos, J. B. & Larson, A. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. *Science* **301**: 961-964.

Hu, Y. & Cardoso, G. C. 2010.Which birds adjust the frequency of vocalizations in urban noise? *Anim. Behav*. **79**: 863-867.

Kirschel, A. N. G., Blumstein, D. T., Cohen, R. C., Buermann, W., Smith, T. B. & Slabbekoorn, H. 2009. Birdsong tuned to the environment: green hylia song varies with elevation, tree cover, and noise. *Behav. Ecol.* **20**: 1089-1095.

Klicka, J., Voelker, G. & Spellman, G. M. 2005. A molecular phylogentic analysis of the “true thrushes” (Aves: Turdinae). *Mol. Phylogenet. Evol.* **34**: 486-500.

Lovette, I. J., Pérez-Emán, J. L., Sullivan, J. L., Banks, R. C., Fiorentino, I., Córdoba-Córdoba, S. *et.al*. 2010. A comprehensive multilocus phylogeny for the Wood-Warblers and a revised classification of the Parulidae (Aves). *Mol. Phylogenet. Evol.* **57**: 753-770.

Lovette, I, J. & Bermingham, E. 1999. Explosive speciation in the New World *Dendroica* warblers. *Proc. R. Soc. Lond. B* **266**: 1629-1636.

Marler, P. & Pickert, R. 1984. Species-universal micro-structure in the learned song of the swamp sparrow, *Melospiza georgiana*. *Anim. Behav*. **32**: 673-689.

Mason, N. 2012. Song complexity and its evolutionary correlates across a continent-wide radiation of songbirds. MSc thesis, San Diego State University.

Nemeth, E., Winkler, H. & Dabelsteen, T. 2001. Differential degradation of antbird songs in a neotropical rainforest: adaptation to perch height? *J. Acoust. Soc. Am.* **110**: 3263-3274.

Nowicki, S. & Searcy, W. 2004. Song function and the evolution of female preferences: Why birds sing and why brains matter. *In*: Behavioral Neurobiology of Birdsong (H. P. Zeigler & P. Marler), pp 1-37. New York Academy of Sciences Press, New York.

Ohlson, J. I., Fjeldså, J. & Ericson, P. G. P. 2013. Molecular phylogeny of the manakins (Aves: Passeriformes: Pipridae), with a new classification and the description of a new genus. *Mol. Phylogen. Evol.* **69**: 796-804.

Ohlson, J., Fjeldså, J. & Ericson, P. G. P. 2008. Tyrant flycatchers coming out in the open: phylogeny and ecological radiation of Tyrannidae (Aves, Passeriformes). *Zool. Scripta* **37**: 315-335.

Phillimore, A. B. & Price, T. D. 2008. Density-dependent cladogenesis in birds. *PLOS Biol.***6**: 483-489.

Podos, J., Huber, S. K. & Taft, B. 2004. Bird Song: the interface between evolution and mechanism. *Annu. Rev. Ecol. Evol. Syst.* **35**: 55-87.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's Finches. *Nature* **409**: 185-187.

Podos, J. 1997. A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). *Evolution* **51**: 537-551.

Price, T. 1998. Sexual selection and natural selection in bird speciation. *Phil. Trans. R. Soc. Lond. B* **353**: 251-260.

Price, J. J. & Lanyon, S. M. 2002. Reconstructing the evolution of complex bird song in the oropendolas. *Evolution* **56**: 1514-1529.

Price, J. J. & Lanyon, S. M. 2004. Patterns of song evolution and sexual selection in the oropendolas and caciques. *Behav. Ecol.* **15**: 485-497.

Price, J. J.**,** Friedman, N. R, & Omland, K. E. 2007. Song and plumage evolution in the New World orioles (*Icterus*) show similar lability and convergence in patterns. *Evolution* **61**: 850-863.

Prum, R. O. 1998. Sexual selection and the evolution of mechanical sound production in manakins (Aves: Pipridae). *Anim. Behav.* **55**: 977-994.

Rabosky, D & Lovette, I. J. 2008. Density-dependent diversification in North American wood warblers. *Proc. R. Soc. B.* **275**: 2363-2371.

Schuster, S., Zollinger, S. A., Lesku, J. A. & Brumm, H. 2012. On the evolution of noise-dependent vocal plasticity in birds. *Biol. Lett.* **8**, doi: 10.1098/rsbl.2012.0676.

Slabbekoorn, H. & Smith, T. B. 2002. Bird song, ecology and speciation. *Philos. Trans. R. Soc. Lond. B* **357**: 493-503.

Slater, P. J. B. 1989. Bird song learning: causes and consequences. *Ethol. Ecol. Evol.* **1**:19-46.

Snow, D. 2004. Family Pipridae (Manakins). *In:* Handbook of the Birds of the World, Vol. 9. Cotingas to Pipits and Wagtails (J. del Hoyo, A. Elliott & J. Sargatal), pp. 110-169. Lynx Edicions, Barcelona.

Suthers, R. A. & Hector, D. H. 1982. Mechanism for the Production of Echolocation Clicks by the Grey Swiftlet, *Collocmias podiopygia*. *J. Comp. Physiol.* **148**: 457-470.

Suthers, R. A. & Hector, D. H. 1985. The physiology of vocalization by the echolocating oilbird, *Steatornis caripensis*. *J. Comp. Physiol.* **56**: 243-266.

Seddon, N. 2005. Ecological adaptation and species recognition drives vocal evolution in Neotropical suboscine birds. *Evolution* **59**: 200-215.

Tobias, J. A. & Seddon, N. 2009. Sexual selection and ecological generalism are correlated in antbirds. *J. Evol. Biol.* **22**: 623-636.

Tobias, J. A., Aben, J., Brumfield, R.T., Derryberry, E., Halfwerk, W., Slabbekoorn, H. et al. 2010. Song divergence by sensory drive in Amazonian birds. *Evolution* **64**: 2820-2839.

Zimmer, K. & Isler, M. 2003. Family Thamnophilidae (Typical Antbirds). *In*: Handbook of the Birds of the World, Vol. 8. Broadbills to Tapaculos (J. del Hoyo, A. Elliott & J. Sargatal), pp. 448-681. Lynx Edicions, Barcelona.

Weir, J. T., Wheatcroft, D. & Price. T. 2012. The role of ecological constraint in driving the evolution of avian song frequency across a latitudinal gradient. *Evolution* **66**: 2773-2783.

Weir, J. T. & Wheatcroft, D. 2011. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Proc. R. Soc. Lond. B.* **278**: 1713-1720.

Weir, J. T. 2006. Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. *Evolution* **60**: 842-855.

Wiley, R. H. 1991. Associations of song properties with habitats for territorial oscine birds of eastern North America. *Am. Nat*. **138**: 973-993.

Wiley, R. H. & Richards, D. G. 1982. Adaptations for acoustic communication in birds: sound transmission and signal detection. *In*: *Acoustic Communication in Birds* (D. E. Kroodsma & E. H. Miller), pp. 131-181. Academic Press, New York and London.

Wilkins, M. R., Seddon, N. & Safran, R. J. 2013. Evolutionary divergence in acoustic signals: causes and consequences. *Trends Ecol. Evol.* **28**: 156-166.

FIGURE CAPTIONS

Figure 1. Spectrogram illustrating seven quantitative song measurements used in the present study (*Myrmecyza laemosticta* XC3303). SD: song duration (s), the duration from the beginning of the first element (note) to the end of the last element in the song; (NN): number of elements (notes) detected within the song; ER: element rate (s), the average duration of elements (notes) within a song; Fmax: Maximum frequency (kHz), the highest frequency across the entire song; Fmin: Minimum frequency (kHz), the lowest frequency across the entire song; FB: Frequency Bandwidth (KHz), the range in frequency values within a song. NDN: Number of different notes types found within a phrase. This spectrogram shows three different notes types signed by “a”, “b” and “c”. PF: Peak frequency also was measured but was omitted here because it is impossible visually to determine in which pixel within a spectrogram represent the higher sound energy, which characterizes peak frequency.

Figure 1.

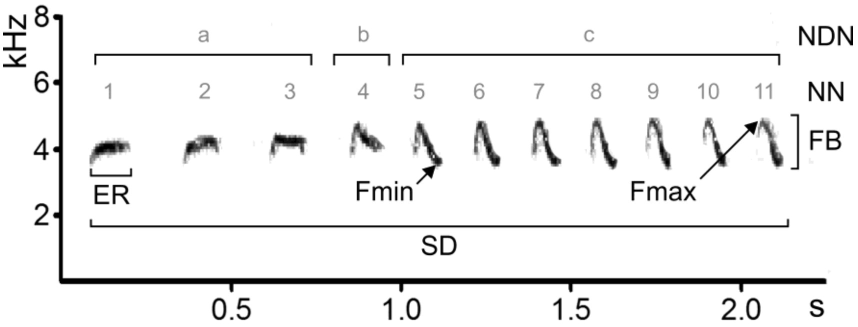
kHz
8
6
4
2
a
b
c
NDN
1
2
3
4
5
6
7
8
9
10
11
NN
FB
ER
Fmin
Fmax
SD
0.5
1.0
1.5
2.0
s

Table 1.Number of species and recordings per family with mean and standard deviation of each song trait analysed in this study.

	Thamnophilidae	Tyrannidae	Pipridae	Parulidae	Turdidae
Number of species	122	77	35	75	27
Number of recordings	842	684	220	414	1013
Mean number of recordings per species (range)	6.902 (1-31)	8.883 (1-40)	6.286 (1-22)	15.333 (1-30)	13.507 (1-27)
Song duration (SD) (s)	3.05 ± 2.88	1.18 ± 0.86	0.71 ± 0.57	2.22 ± 1.30	2.66 ± 2.69
Number of notes (NN)	15.03 ± 10.07	9.96 ± 10.04	3.56 ± 2.96	15.63 ± 10.50	8.92 ± 12.86
Element rate (ER)	0.27 ± 0.19	0.16 ± 0.16	0.21 ± 0.34	0.17 ± 0.05	0.28 ± 0.16
Peak frequency (PF) (Hz)	2988.99 ± 1300.35	4223.66 ± 1436.26	3694.72 ± 1565.18	5262.17 ± 1104.91	3360.54 ± 853.66
Maximum frequency (Fmax) (Hz)	3647.70 ± 1576.18	5293.19 ± 1800.11	4670.72 ± 1755.39	7481.44 ± 1451.69	5512.43 ± 1721.71
Minimum frequency (Fmin) (Hz)	2002.69 ± 1009.00	2225.09 ± 1178.88	2141.18 ± 1231.83	3114.84 ± 1024.71	2020.70 ± 556.23
Frequency bandwidth (FB) (Hz)	1645.01 ± 827.61	3068.11 ± 1413.95	2529.54 ± 1138.22	4366.60 ± 1162.09	3491.74 ± 1673.46
Number of different note types (NDN)	1.90 ± 0.61	2.25 ± 0.97	1.74 ± 0.82	4.75 ± 3.78	4.93 ± 4.51

Table 2. Principal component analysis with correlation matrix on song traits of the five studied families.

	Thamnophilidae			Tyrannidae			Pipridae			Parulidae				Turdidae		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC4	PC1	PC2	PC3
Song duration (SD)	0.13	-0.26	0.74	0.19	0.38	-0.62	-0.16	0.60	0.31	-0.07	0.58	0.17	0.28	0.50	-0.14	-0.10
Number of notes (NN)	-0.01	0.43	0.65	-0.02	0.57	-0.04	-0.26	0.32	-0.48	-0.25	0.48	0.37	-0.17	0.43	-0.30	-0.36
Element rate (ER)	0.13	-0.63	0.08	0.23	-0.10	-0.67	-0.09	0.50	0.53	0.29	-0.05	-0.3	0.77	0.25	-0.01	0.57
Peak frequency (PF)	-0.52	-0.11	0.04	-0.52	-0.10	-0.25	-0.52	-0.18	0.13	-0.53	-0.21	0.07	0.15	-0.38	-0.39	-0.13
Maximum frequency (Fmax)	-0.53	-0.08	0.07	-0.55	0.00	-0.17	-0.53	-0.19	0.08	-0.55	-0.08	-0.26	0.12	-0.21	-0.55	0.20
Minimum frequency (Fmin)	-0.48	-0.18	0.03	-0.35	-0.43	-0.24	-0.39	-0.32	0.20	-0.39	-0.28	0.41	0.40	-0.38	-0.09	-0.52
Frequency bandwidth (FB)	-0.43	0.07	0.09	-0.41	0.36	-0.02	-0.4	0.06	-0.10	-0.34	0.14	-0.69	-0.20	-0.09	-0.53	0.38
Number of diff. note types (NDN)	-0.07	0.54	-0.05	-0.21	0.44	0.15	-0.2	0.34	-0.56	-0.07	0.54	-0.16	0.26	0.40	-0.38	-0.26
Eigenvalue	3.46	1.83	1.30	3.15	2.02	1.19	3.26	1.97	1.54	2.96	2.41	1.26	1.02	3.14	2.65	1.14
Explained variance (%)	43.3	22.8	16.3	39.4	25.3	14.8	40.8	24.6	19.3	36.9	30.2	14.5	12.7	39.3	33.2	14.2

Table 3. Fit of the models of evolution on principal components of the songs traits, of five studied families. WN: White Noise, BM: Brownian motion, OU: Ornstein-Uhlenbeck, and EB: Early burst. Numbers in bold indicate the model with the lowest AICc. See text for details.

		WN		BM			OU			EB		
Family	PC	lnL	AICc	lnL	AICc		lnL	AICc	α	lnL	AICc	A
Thamnophilidae	PC1	-248.37	500.85	-181.25	**368.71**	0.96	-221.18	448.56	3.27	-221.18	448.56	6.55
	PC2	-209.37	422.85	-203.07	412.35	0.55	-200.49	**407.19**	49.96	-200.49	**407.19**	99.92
	PC3	-188.98	382.06	-169.57	**345.35**	0.91	-179.51	365.22	18.64	-179.51	365.22	37.28
Tyrannidae	PC1	-152.95	**310.07**	-153.59	313.51	0.18	-159.13	324.58	25.00	-159.13	324.58	50.00
	PC2	-135.93	**276.03**	-136.86	280.05	0.00	-146.94	300.21	15.70	-146.94	300.21	31.40
	PC3	-115.39	**234.95**	-115.02	236.38	0.00	-130.81	267.96	40.64	-130.81	267.96	81.27
Pipridae	PC1	-69.86	144.11	-67.59	141.98	0.77	-66.88	140.55	0.00	-66.56	**139.92**	32.85
	PC2	-61.03	**126.44**	-61.38	129.57	0.00	-63.23	133.27	19.80	-63.23	133.27	39.59
	PC3	-56.73	**117.84**	-57.11	121.01	0.00	-59.01	124.82	15.31	-59.01	124.82	30.62

Parulidae	PC1	-146.60	297.38	-134.68	**275.71**	0.93	-135.21	276.76	8.40	-135.21	276.76	16.80
	PC2	-138.94	282.05	-123.87	254.09	0.99	-120.90	**248.14**	16.14	-120.90	**248.14**	32.29
	PC3	-111.45	**227.08**	-111.58	229.50	0.00	-114.11	234.56	21.03	-114.11	234.56	42.07
	PC4	-106.46	217.08	-104.45	**215.24**	0.68	-105.79	217.91	17.66	-105.79	217.91	35.32
Turdidae	PC1	-53.26	111.05	-47.49	**102.07**	0.87	-49.47	106.03	1.22	-49.47	106.03	2.45
	PC2	-50.97	**106.47**	-51.28	109.65	0.00	-56.97	121.04	10.73	-56.97	121.04	21.47
	PC3	-39.53	**83.58**	-39.47	86.04	0.89	-40.73	88.55	0.73	-40.73	88.55	1.45

5. ARTIGO III

Morphological constraints in song structure: a comparison between oscine and suboscine birds

Viviane Deslandes & Marcio R. Pie

Capítulo formatado de acordo com a instrução aos autores da revista "Journal of Avian Biology".

Title: Morphological constraints in song structure: a comparison between oscine and suboscine birds

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5.1. Abstract

Body size and beak morphology are characteristics known to affect acoustic structure in birdsongs. Birds cannot efficiently produce sound frequencies higher than those allowed by their body size or their sound producing apparatus. Therefore, there should be a negative relationship between song frequency and body size (frequently represented by body mass). Song production also is affected by beak morphology due to tradeoff in how rapidly and widely a bird can open and close the beak while singing, leading to a negative relationship between frequency bandwidth (FB) and song rate (SR), known as vocal deviation. In this study we investigated the generality of morphological constraints on birdsong evolution in suboscine (Thamnophilidae, Tyrannidae and Pipridae) and oscine (Parulidae and Turdidae) birds. We tested whether there is a triangular negative relation between FB and SR in these families, using both upper bound and quantile regression methods. We also tested the beak morphology and body mass affect directly FB and SR. We used principal component analysis to summarize four measures of beak morphology and Phylogenetic Generalized leas square (PGLS) regression to test these relations. Upper bound results were affected by intervals established in song rate. Using interval of 1 Hz in song rate, only Pipridae exhibited the expected negative relation between FB and SR, but using 2 Hz, Thamnophilidae, Pipridae and Turdidae showed significant results. The best fit of PGLS models were that included the interaction between beak morphology and log of body mass. Beak morphology and body mass affected FB and SR in Thamnophilidae and Parulidae. In Thamnophilidae, small beak and small body mass produces faster rates and broad frequency bandwidth, consistent with the hypothesis of constraints on sound production. In Parulidae, the positive interaction between beak size and body size

exhibited the opposite pattern expected by constraint hypotheses, indicating that heavier birds with larger beaks produce higher FB values.

5.2. Introduction

Phenotypic traits, such those involved in animal communication, may be subject to an array of competing and/or complementary evolutionary pressures (Endler 1993). Particularly in the case of birdsongs, evidence supports the influence of historical factors and intra as well as interspecific variation in morphological, ecological and behavioral traits in shaping song evolution (Podos and Warren 2007). Two morphological characteristics that are known to affect acoustic structure in birdsongs are body size and beak morphology. For instance, birds cannot efficiently produce sound frequencies higher than those allowed by their body size or their sound producing apparatus (i.e. syrinx) (Bradbury andVehremcamp 1998). As a consequence these physical constraints, a negative relationship between song frequency and body size has been recovered in a variety of taxa, including both passerines (Wallshlager 1980, Ryan and Brenowitz 1985, Badyaev and Leaf 1997, Mason 2012), and non-passerines (Ryan and Brenowitz 1985, Tubaro and Mahler 1998, Bertelli and Tubaro 2002).

In songbirds, the beak movements have an important function to sound production. Motor constraints on beak movements during song production result in a trade-off between how rapidly and how widely a singing bird can open and close the beak, leading to a trade-off between song rate (number of syllable repetition divided by song duration) and frequency bandwidth. The relationship between song rate and frequency bandwidth is defined as vocal deviation, whereas vocal performance corresponds to the bird’s ability to meet the trade-off between song rate and frequency bandwidth (Podos 1997, 2001). Hence, beak morphology and function may cause divergence in vocal performance abilities and consequently on song structure (Podos and Nowicki 2004). A series of studies have pointed out the existence of a relationship between vocal performance and beak size (Podos 2001, Huber and Podos 2006, Derryberry 2009, Derryberry et al. 2012). In addition, mechanical simulations of jaw movements suggest that larger beaks are more constrained in their ability to produce rapid movements required for fast and broad-band trills than in the case of birds with smaller beaks (Nowicki et al.1992, Podos and Nowicki 2004). For instance, in Darwin's finches, birds with larger beaks and body sizes exhibit songs with lower rates of syllable repetition and narrower frequency bandwidths when compared to smaller birds (Podos 2001, Huber and Podos 2006). The same relationship was found in swamp sparrows (*Melospiza georgiana*), in which the increase in beak size corresponded to a decrease on vocal performance (Ballentine 2006).

These patterns found in songbirds are also reported in studies with suboscines. Derryberry et al. (2012) used phylogenetic comparative methods to investigate the relationship between beak size and song performance in Neotropical woodcreepers. These authors analyzed a large dataset, including 52 species and 46 subspecies, and found a positive relationship between beak size and vocal deviation across species, indicating that birds with large beaks produce songs with low performance. On the other hand, in thamnophilids, after controlling for the effects of phylogenetic relatedness and body size, beak size did not predict variation in acoustic frequencies of loudsongs, but is instead strongly related to a temporal song pattern (Seddon 2005).

Most studies relating beak morphology to bird song evolution were carried out with songbirds (Podos et al. 2009, Cardoso and Hu 2011, Wilson et al. 2014). A few other bird taxa have been investigated (Palacios and Tubaro 2000, Derryberry et al. 2012), and in these taxa only few studies used comparative methods to control the effect of shared history between species (Derryberry et al. 2012). Therefore, one should have caution when generalizing the established patterns in songbirds to other bird taxa, given that sound production in these birds is different from suboscines. In this study we use phylogenetic comparative methods to test the relation between song traits (frequency bandwidth and song rate) and morphological traits (beak morphology and body size) in suboscine families (Thamnophilidae, Tyrannidae and Pipridae) and oscine families (Parulidae and Turdidae). We have chosen these families due to their broad variation in beak morphology and song structure (Fig. 1), and the availability of recently published molecular phylogenies. Our goal was to compare the results between suboscine and oscine birds to verify the generality of hypothesis on morphology as a constraint to birdsong evolution. In particular, we asked the following questions: (i) Do the studied taxa show a negative relation between frequency bandwidth and song rate reported for several species (e.g. Podos 1997, Derryberry 2009, Derryberry et al. 2012, Ballentine, 2006)?; (ii) Do birds that exhibit larger beaks will produce songs with lower frequency bandwidth and slower song rate values?; (iii) Do smaller birds feature songs with higher frequencies when compared to larger birds?

5.3. Methods

Song data

We measured a total of 3064 songs from five families: Thamnophilidae, Tyrannidae, Pipridae, Parulidae and Turdidae (Table 1). Recordings were obtained from the following collections: Instituto de Investigación de Recursos Biológicos Alexander Von Humboldt (Colombia), Acervo Neotropical Elias Coelho (Universidade Federal do Rio de Janeiro, Brazil), Macaulay Library (Cornell University, EUA), XENO-CANTO database (http://www.xeno-canto.org), and private song collections of Jeremy Minns and Christian Borges Andretti. When possible, recordings were sampled throughout the entire range of each species to consider intraspecific variation in song. Recordings from different localities or dates were assumed to represent different individuals.

Only songs with sample rate and resolution higher that 22.050 Hz and 16 bits were analysed. The spectrograms were generated in the software AVISOFT SAS Lab Pro 5.1, with the following specifications: Window: Hamming, FFT: 256, Frame Size: 100%, and Overlap: 88%. Song duration and number of notes were estimated using automatic parameters "two thresholds", fitting the threshold to each song independently to visualize the best cut-off value in the power spectrum graphic and to select all notes in each recording, while excluding the noise in the background. Maximum and minimum frequencies (F_{max} and F_{min}, respectively), were estimated using the manual cursor, given songs showed considerable variation in relation to the presence of harmonics, and as a consequence, the accurate automatic estimation of these parameters could be compromised. Frequency bandwidth (FB) was calculated subtracting values of Maximum frequency (the highest frequency across the entire song) from Minimum frequency (the lowest frequency across the entire song) to each recording in our sample. Song rate (SR) of each recording was estimated by dividing the number of notes by song duration.

Morphological data

We obtained beak measures from specimens housed at Museu de Zoologia da USP (MZUSP), American Museum of Natural History (AMNH), Cornell University Museum of Vertebrates (CUMV) and Smithsonian National Museum of Natural History (NMNH). We measured four linear variables that represent beak size (Fig. 1f): (1) length of exposed culmen (LEC): the point at which the feathers of forehead in their natural position cease to hide the culmen in a straight line to the tip of the culmen; (2) length of beak from gape (LBG): the length in a straight line from the tip of the maxilla to the corner of the mouth; (3) height of the beak at nostrils (HB): measure from the culmen to the lower edge of the mandible at the anterior end of the nostrils and (4) width of beak (WB): measured vertically at the level of the anterior border of nostrils (Baldwin et al.1931, Derryberry et al. 2012). All measures were made using digital calipers and when possible, ten individuals (five males and five females) were measured for each species. We used the mean between sexes to characterize the variation in beak morphology per species. Principal component analysis using correlation matrix was performed on the logarithmized mean of beak measures (Table S1) within species to reduce the data dimensionality. The broken stick criterion was used to retain the principal component axes for later analysis.

Information about body mass by species was compiled from Dunning (2008) and in del Hoyo et al. (2003 - 2005 and 2010). We used average values when only ranges were reported or when data to male and female were reported separately. Body mass data also was log-transformed for later analyses. There are no clear predictions about how variation in body mass might shape the expression of vocal deviations (Podos 2001), but because body mass might constrain song frequency, we included it as a covariate in our analyses (Table S1).

Analysis

For many species of oscine birds there is a triangular inverse relationship between song rate and frequency bandwidth with a clear trade-off: while songs with low rate may have narrow or broad frequency bandwidths, however, fast rate songs are restricted to narrower bandwidths (Podos 1997). Upper bound regression -the standard method to estimate vocal performance - consists in dividing the range of song rate values into equal intervals, regressing only the maximum frequency bandwidth values in each interval (Podos 1997, Wilson et al. 2014). The orthogonal deviation from this upper limit is called vocal deviation, which is a measure of vocal performance (the greater the deviation from the upper limit, the lower is the vocal performance), while the proximity from the upper limit means a high vocal performance (Podos 2001). However, upper bound regressions are sensitive to sample size, given that analyses with small datasets can find a negative relation between frequency bandwidth and rate that is biased by sample size (Wilson et al. 2014). Although our dataset encompasses a large number of species, few of them are represented with sufficient number of recordings to perform upper bound regressions individually (with 20 recordings being the minimum, J. Podos pers.comm). Thus, to answer our first question about whether the studied taxa show the same triangular negative relation between frequency bandwidth and song rate, we used two alternative approaches: we fitted both upper bound and quantile regressions. To perform the upper bound regressions we grouped all species of each family. Quantile regressions were performed in QUANTREG 5.05 package (Koenker 2013) on FB and SR original data of each family. We use $\tau = 0.90$ in our quantile regressions because previous studies suggest that its slope can be estimated precisely, being resistant to outliers while accurately estimating the expected trade-off found near the upper boundary of a triangular distribution (Wilson et al. 2014).

Given that we found conflicting results depending on the used method to estimate vocal deviation (upper bound using different intervals in song rate and quantile regression), we also used Phylogenetic Generalized Least Square regression (PGLS) to investigate directly the relation of beak morphology and body size on frequency bandwidth and song rate (second and third questions in this study). We used the average value of frequency bandwidth and song rate per species and compared the fit of six models based on AIC values. Frequency bandwidth was the dependent variable in the models 1 to 3. In model 1, the principal component scores of the beak and log of body mass were the independent variables; in model 2, only the principal component of the beak was the independent variable; and in model 3, the independent variables included the interaction between the principal components of the beak and log of body mass. Models 4 to 6 followed the same scheme of models 1 to 3, only replacing frequency bandwidth for song rate as dependent variable. In all models the phylogenetic relationships were considered using the phylogenetic variance/covariance matrix among species, as fitted by BM model.

5.4. Results

Results of principal component analyses of beak measurements are shown in Table 2. PC1 explained 75-92% of the variance and was the only component retained for further analyses based on the broken-stick criterion in four out of five studied families. Given that the loadings on PC1 had the same sign and similar magnitudes, scores on this PC were interpreted as reflecting a general measure of beak size. The only exception was Pipridae, where the first two PCs had similar explained variances (49.9% and 39.9%, respectively). In particular, loadings on the first PC in piprids can be interpreted as a measure of relative beak depth, given the negative relationship between HB and LBG + WB, whereas PC2 reflected mostly the relative magnitude of LEC and HB.

We found conflicting results on tests of a triangular and negative relationship between frequency bandwidth and song rate depending on the method used and the interval established in song rate for the upper bound regression calculation. Using upper bound regression with an interval of 1 Hz in song rate, we found the predicted negative relation between FB and SR only in piprid songs (Fig. 2). However, when the used interval in song rate was 2 Hz, Thamnophilidae, Pipridae and Turdidae exhibited significant results (Fig. 2b, f and j). Conversely, the quantile regression between frequency bandwidth and song rate showed a positive and significant relation in all families, exception for Pipridae (Fig. 3, Table 3).

The PGLS with the best fit were those that included the interaction between principal component of the beak and log of body mass. In particular, models 3 and 6 resulted in the lowest AIC values in all families (Table 4). The fit of all PGLS models are presented in Table S2. A significant effect of beak morphology and body size on frequency bandwidth and song rate was only detected in Thamnophilidae and Parulidae. In Thamnophilidae, frequency bandwidth was inversely related to beak morphology and body size, i.e smaller birds produce higher frequency bandwidth values and smaller beaks produce a broader frequency bandwidth, according with the constraint hypothesis to sound production. Conversely, song rate showed a positive relation with beak morphology and body size: larger beaks and body size were associated to faster song rates. However, the interaction between beak morphology and body size was inversely related to song rate, indicating that, when these variables are considered together, smaller beaks and body size produces the faster rates, again consistent with the hypothesis of constraints on sound production.

Parulidae also exhibited a negative relation between frequency bandwidth and beak morphology. However, when the interaction between beak morphology and body size was taken into account, we found a positive and significant relation between these variables, indicating that heavier birds with larger beaks produce higher frequency bandwidth values, in contrast with the hypothesis of constraints on sound production (Table 4).

5.5. Discussion

In the past years an increasing number of studies found support for the original hypothesis of a relationship between beak size and vocal performance (Podos 1997, 2001), which shows an inverse relation between vocal performance and beak size: larger beaks are more constrained in their ability to produce rapid movements, required for fast and broad-band signals (Podos 2001, Huber and Podos 2006, Derryberry 2009, Derryberry et al. 2012). However, all these studies estimated vocal performance based on upper bound regressions method, which has been recently shown to be biased when sample sizes are small. Wilson et al. (2014) analyzed 70 datasets using three alternative methods to estimate vocal deviation: traditional upper bound method, quantile regression and upper bound regression correcting by sample size. Surprisingly, half of studies that found significant estimates of performance trade-offs based in upper bound methods had low sample size, leading to false positives. Thus, they concluded that among the three compared methods, quantile regression is the most reliable to estimate vocal deviation, because this method is robust to use with small datasets and not use any data transformation.

In this study we found conflicting results when using upper bound and quantile regression methods. We found more significant results when using interval of 2 Hz in song rate in upper bound regressions than using 1 Hz (Fig 2). This result suggests that upper bound regressions indeed are highly sensitive to values used to divide song rate (or trill rate), at least in these studied families. Given that the relation between FB and SR changes according to the established interval in song rate, we argue that it seems more appropriate to use quantile regression on the original data to investigate this relationship. Using this method, data transformation is unnecessary and the results are independent of sample size, allowing one to use the entire dataset, and consequently incorporating a higher number of species in the analysis, which is more interesting in comparative studies that seek general patterns when comparing several taxa. Furthermore, our quantile regression results exhibited the opposite pattern predicted by vocal performance hypothesis suggesting that at least in four analyzed taxa, most species seem not to have experienced constraints in the production of fast and broad bandwidth signals. One possible explanaiton is that song structure in these families does not present a sufficient fast rate to cause a trade-off, and conversely, allowed the coexistence of high values of both FB and SR or yet, that selection on vocal performance in these families happened in other song variables not analyzed in this study (Wilson et al 2014).

Given that we found conflicting results using different methods to estimate vocal deviation (upper bound vs quantile regression) and that the major question in this study is about the influence of beak morphology and body size on song features (specifically frequency bandwidth and song rate), we considered PGLS the most appropriate method to answer our question. Using this approach, we showed two main points: first, beak morphology and body size should not be dissociated in studies on morphology acting as a constraint in birdsong. All the best models based on lowest AIC values were those that presented the interaction between these variables (Table 4). Second, the relationship between song features and beak morphology, and body size is not consistent among studied taxa. We found significant results of beak morphology and body size affecting FB and SR in two of the five studied families (Thamnophilidae and Parulidae).

In Thamnophilidae we found support for the inverse relation between frequency bandwidth and body size, and beak size. Seddon (2005) found similar results in relation to song structure and body size in this family, but in contrast to our results, in her study beak morphology did not show a significant effect on song frequency. Her argument to explain this result is that the variation in beak length in this family is small when compared to other suboscines taxa, in which a significant and negative correlation between beak morphology and song frequency was detected. Consequently, in Thamnophilidae, body mass is more important in determining song frequency when compared to beak morphology (Seddon 2005). However, our results evidenced that even the small variation in beak size in antbirds is sufficient to generate the inverse relation between beak size and song frequency found in other suboscine family (Dendrocolaptidae) (Palacios and Tubaro 2000, Derryberry et al. 2012). We agree with Seddon’s argument that body mass is important to determine song frequency in antbirds, yet, the constraint imposed by beak morphology should not be ignored as an important contribution to shape aspects of thamnophilid songs. Moreover, at the time of her Seddon's study, no molecular phylogeny was yet available for antbirds and the phylogenetic information in that study was based on a variety of sources, resulting in a phylogeny without branch lengths. It might be so that the differences between Seddon’s and our results could result from differences in how the phylogenetic information was considered in each study.

In Parulidae, the relation between FB and SR in upper bound regressions was negative, but not significant, contrasting with Cardoso and Hu (2011) results, in which the upper bound regression using intervals of 3 Hz in trill rate showed significant results. As in Thamnophilidae, PGLS results for warblers also showed the inverse relation between FB and beak size. However, body mass does not seem equally important in constraining song frequency in this family as well as beak morphology.

In conclusion, our study added weight to the caution in using upper bound regression as a method to estimate vocal deviation (Wilson et al 2014) and shows that PGLS is an appropriated method to study the role of morphology as a constraint on song structure. In addition, we also showed that the universality of the constraint hypothesis should be considered with caution, because only two taxa here studied (Thamnophilidae and Parulidae) supported their predictions. These findings also reinforce the hypothesis suggested by Servedio et al. (2011) and Derryberry et al. (2012), that beak morphology can be considered a *magic trait* in birds. The more plausible explanation in several studies relating song features and beak morphology is the correlated evolution. In other words, beak morphology changes in response to habitat (foraging niches) and this modification affects song structure. For this reason, future studies asking these questions could also focus in ecological information regarding the species, such as diet and habitat structure, once these factors can directly affect beak morphology and song structure (Seddon 2005).

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5.6. References

Ballentine, B. 2006. Morphological adaptation influences the evolution of a mating signal. -Evolution 60: 1936–1944.

Badyaev, A. V. and Leaf, E. S. 1997. Habitat associations of song characteristics in Phylloscopus and Hippolais Warblers. - Auk 114: 40-46.

Bertelli, S. and Tubaro, P. L. 2002. Body mass and habitat correlates of song structure in a primitive group of birds. - Biol. J. Linnean Soc. 77: 423-430.

Bradbury, J. W. and Vehrencamp, S. L. 1998. Principles of Animal Communication. - Sinauer Associates, Sunderland, MA.

Cardoso, G. C. and Hu, Y. 2011. Birdsong performance and the evolution of simple (rather than elaborated) sexual signals. - Am. Nat. 178: 679-686.

Collar, N. 2005. Family Turdidae (Thrushes). - .*In*: del Hoyo, J., Elliott, A. and Sargatal, J. (eds). Handbook of the Birds of the World, Vol. 10. Cuckoo-shrikes to Thrushes. Lynx Edicions, Barcelona, pp 514-807.

Curson, J. 2010. Family Parulidae (New World Warblers). - *In*: del Hoyo, J., Elliott, A. and Sargatal, J. (eds). Handbook of the Birds of the World, Vol.15. Weaves to New World Warblers. Lynx Edicions, Barcelona, pp. 666-800.

Derryberry, E.P., Seddon, N., Claramunt, S., Tobias, J. A., Baker, A., Aleixo, A. and Brumfield, R. T. 2012. Correlated evolution of beak morphology and song in the Neotropical woodcreper radiation. - Evolution 66: 2784-2797.

Derryberry, E. P. 2009. Ecology Shapes Birdsong Evolution: Variation in Morphology and Habitat Explains Variation in White-Crowned Sparrow Song. - Am. Nat. 174: 24-33.

Dunning, J. B. 2008. CRC Handbook of avian body masses. CRC Press, Taylor & Francis Group, Boca Raton, USA.

Endler, J. A. 1993. Some general comments on the evolution and design of animal communication systems. - Phil. Trans. R. Soc. Lond. B, Biol. Sci. 340: 215-225.

Fitzpatrick, J., Bates, J., Bostwick, K., Caballero, I., Clock, B., Farnsworth, A. et al. 2004. Family Tyrannidae (Tyrant-flycatchers). - *In*: del Hoyo, J., Elliott, A. and Sargatal, J. (eds). Handbook of the Birds of the World, Vol. 9. Cotingas to Pipits and Wagtails. Lynx Edicions, Barcelona, pp. 170-462.

Huber, S. K. and Podos, J. 2006. Beak morphology and song features covary in a population of Darwin's finches (*Geospiza fortis*). - Biological Journal of the Linnean Society 88: 489-498.

Koenker, R. 2013. Package "quantreg". R package vesion 5.05. http://cran.rproject.org/web/packages/quantreg/quantreg.pdf

Mason, Nicholas. 2012. Song complexity and its evolutionary correlates across a continent-wide radiation of songbirds. MSc thesis, San Diego State University.

Nowicki, S., Westneat, M. W. and Hoese, W. J. 1992. Birdsong: Motor function and the evolution of communication. - Seminars in the Neurosciences 4: 385- 390.

Palacios, M. G. and Tubaro, P. L. 2000. Does beak size affect acoustic frequencies in Woodcreepers? - Condor 102: 553-560.

Podos, J. and Warren, P. S. 2007. The Evolution of Geographic Variation in Birdsong. - Advances in the Study of Behavior, 37: 403-458.

Podos, J., Huber, S. K. and Taft, B. 2004. Bird Song: The Interface of Evolution and mechanism. - Annual Review of Ecology, Evolution, and Systematics 35: 55-87.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin’s Finches. - Nature 409: 185-187.

Podos, J. 1997. A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). - Evolution 51: 537- 551.

Ryan, M. J. and Brenowitz, E. A. 1985. The role of Body Size, Phylogeny and Ambient Noise in the evolution of Bird Song. - Am. Nat. 126: 87-100.

Seddon, N. 2005. Ecological adaptation and species recognition drives vocal evolution in Neotropical suboscine birds. - Evolution 59: 200-215.

Snow, D. 2004. Family Pipridae (Manakins). - *In:* del Hoyo, J., Elliott, A. and Sargatal, J. (eds). Handbook of the Birds of the World, Vol. 9. Cotingas to Pipits and Wagtails. Lynx Edicions, Barcelona, pp. 110-169.

Tubaro, P. L. and Mahler, B. 1998. Acoustic frequencies and body mass in New World doves. - Condor 100: 54-61.

Zimmer, K. and Isler, M. 2003. Family Thamnophilidae (Typical Antbirds). - *In*: del Hoyo, J., Elliott, A. and Sargatal, J. (eds). Handbook of the Birds of the World, Vol. 8. Broadbills to Tapaculos. Lynx Edicions, Barcelona, pp. 448–681.

Wallschlager. D. 1980. Correlation of song frequency and body weight in passerine birds. - Experiential 36: 412.

Wilson, D. R., Bitton, P. B., Podos, J. and Mennill, D. J. 2014. Uneven Sampling and the Analysis of Vocal Performance Constraints. - Am. Nat.183: 214-228.

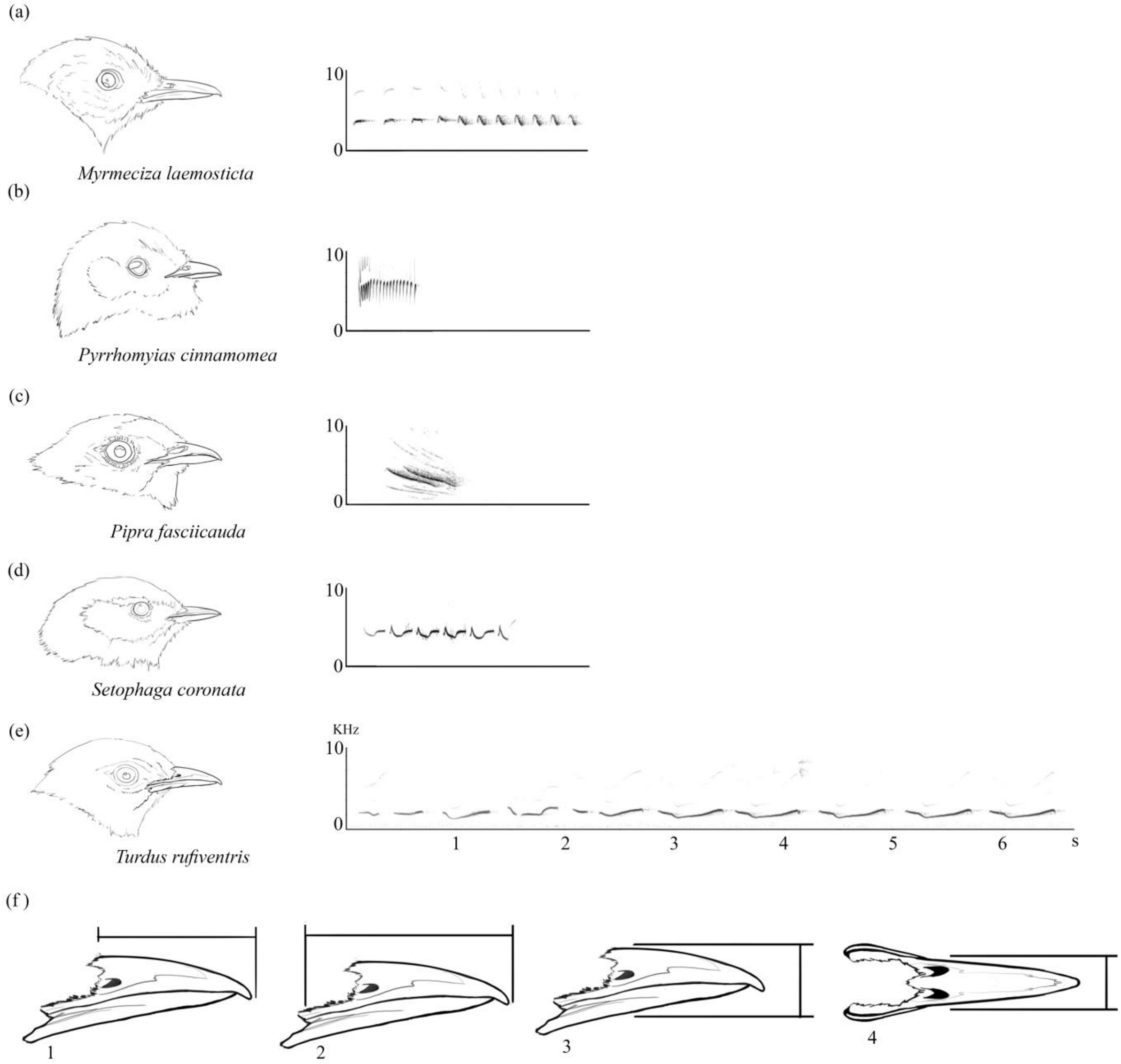
FIGURE CAPTIONS

Figure1. Beak morphology and representative song spectrograms of five selected species of the studied families (a): Thamnophilidae; (b): Tyrannidae; (c): Pipridae; (d): Parulidae and (e): Turdidae. (f): Beak measurements; (f1): length of exposed culmen (LEC); (f2): length of beak from gape (LBG); (f3): height beak at nostrils (HB) and (f4): width of beak (WB).

Figure 2. Upper bound scatterplots of frequency bandwidth (Y axis) and song rate (X axis) of the five studied families. Scatterplots at left show upper bound regressions using bins of 1Hz in song rate while scatterplots at right show upper bound regressions using bins of 2 Hz. (**a)** Thamnophilidae (1 Hz): -34.15x + 3850.58, R^2=0.085, P=0.140; (**b)** Thamnophilidae (2 Hz): -64.27x + 4743.62, R^2=0.327, P=0.025; (**c)** Tyrannidae (1 Hz): -25.59x + 5933.70, R^2= 0.034, P=0.250; (**d)** Tyrannidae (2 Hz): -38.12x + 6760.47, R^2=0.086, P=0.184; (**e)** Pipridae (1 Hz): -91.33x + 5905.62, R^2=0.473, P<0.005; (**f)** Pipridae (2 Hz): -115.83x + 6750.06, R^2=0.678, P< 0.005; (**g)** Parulidae (1 Hz): -16.82x + 6309.48, R^2=0.023, P=0.381; **(h)** Parulidae (2 Hz): -21.36x + 6870.90, R^2=0.060, P=0.326; **(i)** Turdidae (1 Hz): -209.9x + 7697.3, R^2=0.182, P=0.165; (**j)** Turdidae (2 Hz):-338.5x + 9390 R^2=0.654, P=0.051.

Figure 3.Quantile regression using τ=0.90 on the original data of the five studied families. (a): Thamnophilidae; (b): Tyrannidae, (c): Pipridae; (d): Parulidae and (e): Turdidae. All families showed a strong positive relation between frequency bandwidth (Hz) and song rate (Hz), only in Pipridae this relation was insignificant.

Figure 1.


(a)
Myrmeciza laemosticta
10
0
(b)
Pyrrhomyias cinnamomea
10
0
(c)
Pipra fasciicauda
10
0
(d)
Setophaga coronata
10
0
(e)
Turdus rufiventris
KHz
10
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1
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4

Figure 2.

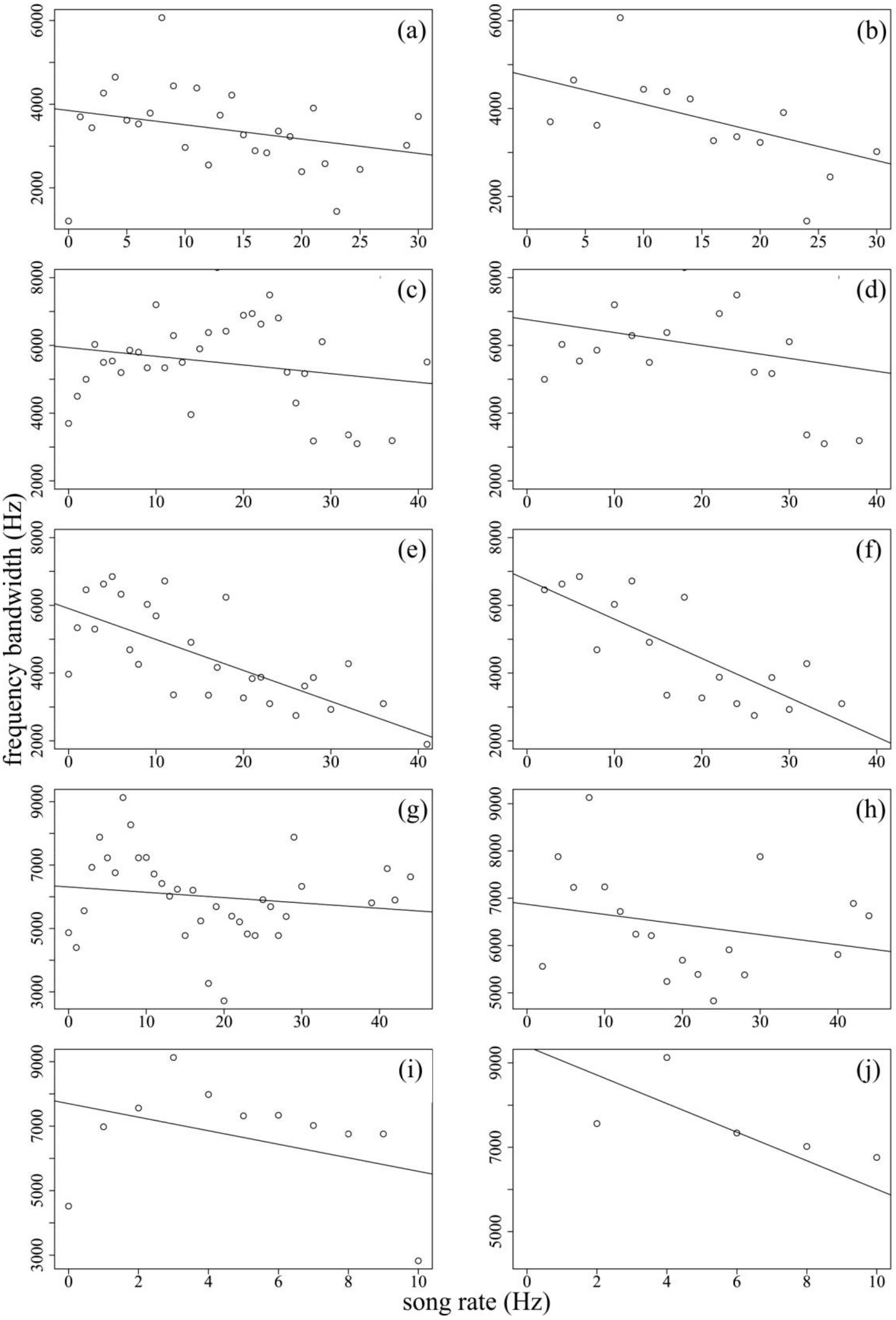
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
frequency bandwidth (Hz)
song rate (Hz)
2000
4000
6000
8000
3000
5000
7000
9000
0
5
10
15
20
25
30
40

Figure 3.

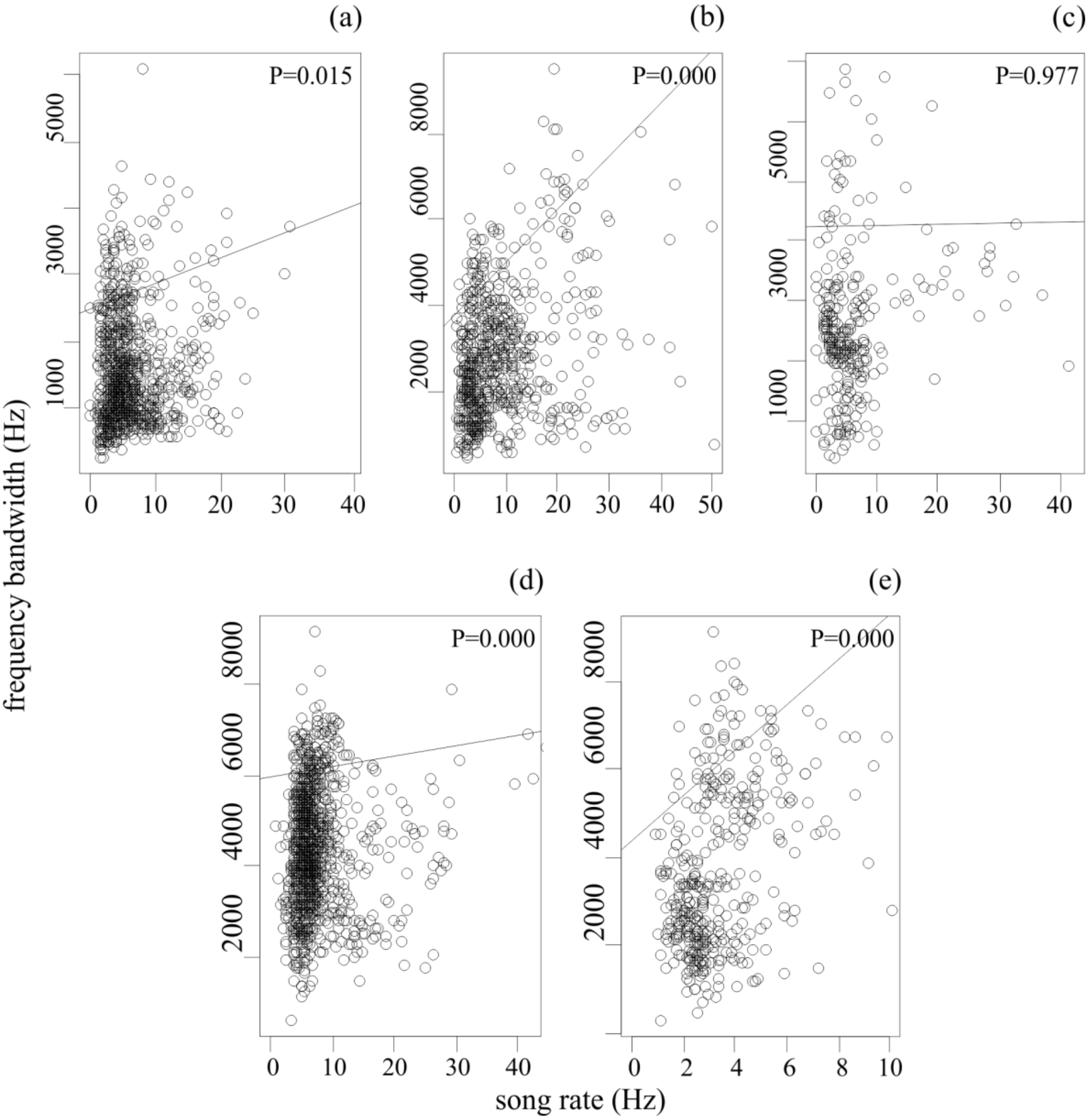
(a)
P=0.015
(b)
P=0.000
(c)
P=0.977
(d)
P=0.000
(e)
P=0.000
frequency bandwidth (Hz)
song rate (Hz)
1000
3000
5000
2000
4000
6000
8000
0
10
20
30
40
50
2
4
6
8

Table 1. Number of species and recordings per family to which there is phylogenetic and morphological data associated in this study, totalizing 318 species and 3064 recordings. NS: number of species; NR: number of recordings; MNRS: mean and range of the number of recordings per species.

Family	NS	NR	MNRS
Thamnophilidae	112	802	7.16 (1-31)
Tyrannidae	74	668	9.02 (1-40)
Pipridae	32	211	6.59 (1-22)
Parulidae	74	998	13.48 (1-27)
Turdidae	26	385	14.80 (1-30)

Table 2. Principal component analysis with correlation matrix on the logarithmized average of the beak measures of the five studied families. Length of exposed culmen (LEC); Length from beak to gape (LBG); Height height beak at nostrils (HB) and width of the beak (WB).

Variable	**Thamnophilidae**	**Tyrannidae**	**Pipridae**		**Parulidae**	**Turdidae**
	PC1	**PC1**	**PC1**	**PC2**	**PC1**	**PC1**
log LEC	0.406	0.486	0.120	-0.718	-0.502	0.535
log LBG	0.397	0.480	-0.891	-0.127	-0.464	0.574
log HB	0.659	0.540	0.270	-0.575	-0.486	0.558
log WB	0.493	0.492	-0.345	-0.371	-0.545	0.272
Eigenvalue	0.028	0.059	0.013	0.010	0.009	0.024
Explained variance (%)	86.9	91.8	49.9	39.9	75.8	83.8

Table 3. Quantile regression between Frequency bandwidth (FB) and Song rate (SR). using $\tau = 0.90$. All families show significant and positive relation between these variables, with exception of Pipridae.

Family		**Value**	**SE**	**t**	**P**
	Intercept	2498.263	111.733	22.359	0.000
Thamnophilidae	Song rate	38.315	15.719	2.438	**0.015**
	Intercept	3747.054	164.461	22.784	0.000
Tyrannidae	Song rate	123.860	14.432	8.582	**0.000**
	Intercept	4214.496	486.859	8.657	0.000
Pipridae	Song rate	2.003	69.905	0.029	0.977
	Intercept	5978.950	87.367	68.435	0.000
Parulidae	Song rate	21.865	4.018	5.442	**0.000**
	Intercept	4378.817	389.131	11.253	0.000
Turdidae	Song rate	511.666	100.890	5.072	**0.000**

Table 4. Phylogenetic generalized least squares of the frequency bandwidth and song rate and PC beak and log of body mass (logMASS) to the five studied families. Only the models with lowest AIC values are shown (all models are available in Table S2 in supplementar material). The models with interaction between beak and body mass were the best fit between all models in all families.

Model 3: FB~PCbeak*logMASS	**Variable**	**Estimated**	**SE**	**t-value**	**p-value**
Thamnophilidae (AIC = 1731.366)	PC1beak	-6802.967	3049.525	-2.231	**0.028**
	logMASS	-817.405	380.325	-2.149	**0.034**
	PC1beak:logMASS	4520.967	2227.220	2.030	**0.045**
Tyrannidae (AIC = 1224.864)	PC1beak	-2915.089	3674.020	-0.793	0.430
	logMASS	-401.442	1742.610	-0.230	0.819
	PC1beak:logMASS	99.878	2173.052	0.046	0.964
Pipridae (AIC = 464.994)	PC1beak	-32939.120	36128.710	-0.912	0.370
	PC2beak	19734.280	22829.500	0.864	0.395
	logMASS	-4548.350	4081.330	-1.114	0.275
	PC1beak:logMASS	27146.290	30569.390	0.888	0.383

	PC2beak:logMASS	-26060.020	22490.070	-1.159	0.257
	PC1beak	-498.138	202.713	-2.457	**0.017**
Parulidae (AIC = 648.139)	logMASS	-36.393	36.982	-0.984	0.329
	PC1beak:logMASS	459.554	177.366	2.591	**0.012**
	PC1beak	9825.996	26719.626	0.368	0.717
Turdidae (AIC = 409.029)	logMASS	-1790.769	6177.715	-0.290	0.775
	PC1beak:logMASS	-4117.457	14671.248	-0.281	0.782
Model 6: SR~PCbeak*logMASS					
	PC1beak	90.130	30.845	2.922	**0.004**
Thamnophilidae (AIC= 739.114)	logMASS	11.197	3.847	2.911	**0.004**
	PC1beak:logMASS	-74.353	22.528	-3.301	**0.001**
	PC1beak	-18.472	24.537	-0.753	0.454
Tyrannidae (AIC = 523.626)	logMASS	-3.332	11.638	-0.286	0.776
	PC1beak:logMASS	9.320	14.513	0.642	0.523
Pipridae (AIC = 185.587)	PC1beak	-131.872	167.608	-0.787	0.439

	PC2beak	64.703	105.910	0.611	0.547
	logMASS	18.238	18.934	0.963	0.344
	PC1beak:logMASS	117.481	141.817	0.828	0.415
	PC2beak:logMASS	-53.938	104.336	-0.517	0.610
Parulidae (AIC = 661.585)	PC1beak	263.657	223.147	1.182	0.241
	logMASS	31.342	40.710	0.770	0.444
	PC1beak:logMASS	-149.617	195.245	-0.766	0.446
Turdidae (AIC = 84.332)	PC1beak	-11.607	16.671	-0.696	0.494
	logMASS	-0.986	3.854	-0.256	0.801
	PC1beak:logMASS	6.961	9.154	0.760	0.455

6. CONCLUSÃO

A abordagem comparativa numa escala macrogeográfica permitiu identificar alguns padrões robustos a respeito da evolução do canto. Apesar de cada táxon apresentar um padrão particular é clara importância da história evolutiva em fornecer o potencial de variação dos cantos, uma vez que a filogenia determina a morfologia e fisiologia das espécies. Além disso, alguns padrões gerais foram evidentes independentemente do táxon analisado: (1) as características do canto relacionadas à freqüência são limitadas pelo ambiente e morfologia. Entretanto, para a maioria das famílias características temporais do canto não mostraram qualquer tipo de limitação, com exceção de SR em Thamnophilidae que foi afetada pela morfologia do bico e massa corporal; (2) em geral os modelos mais simples, sem estrutura de correlação (White noise) ou com uma taxa de evolução constante ao longo dos ramos da filogenia (Brownian motion) apresentaram melhor ajuste às características do canto, indicando que elas podem evoluir de modo mais simples do que o usualmente imaginado; (3) a massa corporal é uma importante característica que limita propriedades dos cantos e deveria ser incluída em qualquer estudo bioacústico comparativo.

Table S1. Logarithmized average of the beak measures and body mass to all species included in this study.

Family	Species	logLEC	logLBG	logHB	logWB	logMASS
Thamnophilidae	Cercomacra_carbonaria	1.200	1.287	0.677	0.890	1.161
Thamnophilidae	Cercomacra_cinerascens	1.232	1.299	0.687	0.860	1.204
Thamnophilidae	Cercomacra_laeta	1.196	1.279	0.624	0.925	1.204
Thamnophilidae	Cercomacra_manu	1.200	1.308	0.657	0.877	1.255
Thamnophilidae	Cercomacra_melanaria	1.190	1.259	0.639	0.870	1.279
Thamnophilidae	Cercomacra_nigrescens	1.198	1.314	0.683	0.943	1.312
Thamnophilidae	Cercomacra_nigricans	1.195	1.301	0.661	0.897	1.211
Thamnophilidae	Cercomacra_serva	1.178	1.270	0.661	0.914	1.204
Thamnophilidae	Cercomacra_tyrannina	1.190	1.308	0.656	0.964	1.23
Thamnophilidae	Cymbilaimus_lineatus	1.296	1.387	0.961	1.103	1.574
Thamnophilidae	Dichrozona_cincta	1.197	1.276	0.598	0.886	1.169
Thamnophilidae	Dysithamnus_leucostictus	1.117	1.313	0.695	1.057	1.305
Thamnophilidae	Dysithamnus_mentalis	1.126	1.209	0.671	0.890	1.179
Thamnophilidae	Epinecrophylla_erythrura	1.085	1.178	0.604	0.784	1.041
Thamnophilidae	Epinecrophylla_fulviventris	1.107	1.216	0.622	0.899	1
Thamnophilidae	Epinecrophylla_haematonota	1.079	1.186	0.619	0.765	0.989
Thamnophilidae	Epinecrophylla_leucophthalma	1.092	1.190	0.615	0.859	0.966
Thamnophilidae	Epinecrophylla_ornata	1.126	1.222	0.614	0.819	0.989
Thamnophilidae	Epinecrophylla_spodionota	1.107	1.217	0.628	0.919	0.989
Thamnophilidae	Formicivora_grisea	1.139	1.218	0.570	0.829	1
Thamnophilidae	Formicivora_rufa	1.122	1.221	0.595	0.832	1.106
Thamnophilidae	Frederickena_unduligera	1.364	1.465	1.035	1.051	1.903
Thamnophilidae	Frederickena_viridis	1.358	1.423	0.999	1.122	1.845
Thamnophilidae	Gymnocichla_nudiceps	1.310	1.367	0.750	0.976	1.484
Thamnophilidae	Gymnopithys_leucaspis	1.246	1.307	0.686	1.022	1.491
Thamnophilidae	Gymnopithys_rufigula	1.251	1.341	0.728	0.900	1.462
Thamnophilidae	Gymnopithys_salvini	1.182	1.272	0.701	0.886	1.413
Thamnophilidae	Herpsilochmus_atricapillus	1.123	1.205	0.611	0.854	1.041
Thamnophilidae	Herpsilochmus_dorsimaculatus	1.149	1.211	0.635	0.848	1
Thamnophilidae	Herpsilochmus_longirostris	1.171	1.264	0.658	0.881	1.114
Thamnophilidae	Herpsilochmus_motacilloides	1.133	1.186	0.583	0.805	1.097
Thamnophilidae	Herpsilochmus_rufimarginatus	1.133	1.186	0.583	0.805	1.051
Thamnophilidae	Herpsilochmus_stictocephalus	1.109	1.236	0.556	0.936	0.942
Thamnophilidae	Hylophylax_naevioides	1.198	1.279	0.641	0.869	1.23
Thamnophilidae	Hylophylax_naevius	1.191	1.250	0.616	0.915	1.097
Thamnophilidae	Hylophylax_punctulatus	1.189	1.250	0.644	0.917	1.079

Thamnophilidae	Hypocnemoides_maculicauda	1.202	1.285	0.560	0.839	1.114
Thamnophilidae	Hypoedaleus_guttatus	1.329	1.412	0.995	1.095	1.589
Thamnophilidae	Mackenziaena_leachii	1.324	1.417	0.977	1.054	1.748
Thamnophilidae	Megastictus_margaritatus	1.221	1.323	0.734	1.033	1.29
Thamnophilidae	Microrhopias_quixensis	1.085	1.240	0.625	0.854	0.978
Thamnophilidae	Myrmeciza_atrothorax	1.185	1.268	0.618	0.855	1.204
Thamnophilidae	Myrmeciza_castanea	1.154	1.240	0.595	0.823	1.217
Thamnophilidae	Myrmeciza_exsul	1.282	1.358	0.717	0.962	1.439
Thamnophilidae	Myrmeciza_ferruginea	1.284	1.362	0.682	0.971	1.423
Thamnophilidae	Myrmeciza_fortis	1.315	1.376	0.840	0.992	1.544
Thamnophilidae	Myrmeciza_goeldii	1.335	1.388	0.802	0.980	1.623
Thamnophilidae	Myrmeciza_hemimelaena	1.160	1.229	0.599	0.890	1.19
Thamnophilidae	Myrmeciza_hyperythra	1.346	1.404	0.798	1.017	1.613
Thamnophilidae	Myrmeciza_immaculata	1.310	1.393	0.793	0.963	1.607
Thamnophilidae	Myrmeciza_laemosticta	1.221	1.332	0.676	0.879	1.38
Thamnophilidae	Myrmeciza_longipes	1.244	1.332	0.671	0.963	1.439
Thamnophilidae	Myrmeciza_melanoceps	1.336	1.381	0.810	1.014	1.58
Thamnophilidae	Myrmeciza_nigricauda	1.279	1.365	0.690	1.045	1.352
Thamnophilidae	Myrmeciza_pelzelni	1.166	1.267	0.591	0.802	1.249
Thamnophilidae	Myrmeciza_squamosa	1.146	1.246	0.559	0.808	1.261
Thamnophilidae	Myrmoborus_myotherinus	1.169	1.260	0.676	0.889	1.279
Thamnophilidae	Myrmochanes_hemileucus	1.209	1.275	0.593	0.817	1.097
Thamnophilidae	Myrmorchilus_strigilatus	1.191	1.274	0.640	0.857	1.389
Thamnophilidae	Myrmornis_torquata	1.325	1.437	0.748	1.047	1.667
Thamnophilidae	Myrmotherula_ambigua	1.095	1.177	0.520	0.760	0.875
Thamnophilidae	Myrmotherula_assimilis	1.123	1.202	0.541	0.814	0.954
Thamnophilidae	Myrmotherula_axillaris	1.120	1.203	0.557	0.816	0.903
Thamnophilidae	Myrmotherula_brachyura	1.073	1.146	0.529	0.748	0.845
Thamnophilidae	Myrmotherula_cherriei	1.127	1.223	0.520	0.754	0.916
Thamnophilidae	Myrmotherula_hauxwelli	1.109	1.211	0.583	0.816	1.021
Thamnophilidae	Myrmotherula_longicauda	1.118	1.201	0.533	0.724	0.929
Thamnophilidae	Myrmotherula_longipennis	1.108	1.195	0.539	0.846	0.954
Thamnophilidae	Myrmotherula_menetriesii	1.113	1.206	0.550	0.816	0.929
Thamnophilidae	Myrmotherula_multostriata	1.110	1.190	0.532	0.790	0.903
Thamnophilidae	Myrmotherula_pacifica	1.129	1.235	0.532	0.827	0.966
Thamnophilidae	Myrmotherula_sclateri	1.123	1.196	0.522	0.763	0.954
Thamnophilidae	Neoctantes_niger	1.240	1.316	0.873	0.991	1.484
Thamnophilidae	Percnostola_lophotes	1.257	1.357	0.769	0.961	1.447
Thamnophilidae	Percnostola_rufifrons	1.234	1.332	0.725	0.873	1.423
Thamnophilidae	Phaenostictus_mcleannani	1.319	1.384	0.835	0.920	1.708
Thamnophilidae	Phlegopsis_erythroptera	1.288	1.371	0.785	0.893	1.732
Thamnophilidae	Phlegopsis_nigromaculata	1.280	1.347	0.783	0.972	1.667
Thamnophilidae	Pithys_albifrons	1.180	1.276	0.680	0.903	1.312

Thamnophilidae	Pygiptila_stellaris	1.276	1.368	0.846	1.065	1.398
Thamnophilidae	Pyriglena_leuconota	1.215	1.308	0.721	0.862	1.491
Thamnophilidae	Rhegmatorhina_hoffmannsi	1.220	1.289	0.727	0.945	1.491
Thamnophilidae	Rhegmatorhina_melanosticta	1.262	1.322	0.743	0.860	1.491
Thamnophilidae	Sakesphorus_canadensis	1.272	1.355	0.839	0.928	1.389
Thamnophilidae	Sakesphorus_luctuosus	1.301	1.388	0.835	1.047	1.477
Thamnophilidae	Sclateria_naevia	1.337	1.406	0.697	0.875	1.391
Thamnophilidae	Taraba_major	1.415	1.466	1.016	1.077	1.778
Thamnophilidae	Terenura_humeralis	1.085	1.177	0.547	0.766	0.892
Thamnophilidae	Thamnistes_anabatinus	1.227	1.313	0.802	0.903	1.161
Thamnophilidae	Thamnomanes_ardesiacus	1.206	1.311	0.715	1.053	1.243
Thamnophilidae	Thamnomanes_caesius	1.196	1.309	0.727	1.017	1.23
Thamnophilidae	Thamnomanes_saturninus	1.225	1.301	0.746	0.998	1.301
Thamnophilidae	Thamnomanes_schistogynus	1.192	1.313	0.720	1.033	1.23
Thamnophilidae	Thamnophilus_aethiops	1.212	1.282	0.809	0.935	1.423
Thamnophilidae	Thamnophilus_amazonicus	1.208	1.295	0.755	0.943	1.279
Thamnophilidae	Thamnophilus_aroyae	1.184	1.277	0.785	0.853	1.301
Thamnophilidae	Thamnophilus_atrinucha	1.253	1.344	0.794	0.885	1.352
Thamnophilidae	Thamnophilus_bridgesi	1.329	1.402	0.841	1.089	1.423
Thamnophilidae	Thamnophilus_caerulescens	1.192	1.288	0.772	0.979	1.324
Thamnophilidae	Thamnophilus_doliatus	1.219	1.314	0.795	0.952	1.431
Thamnophilidae	Thamnophilus_murinus	1.223	1.297	0.783	0.965	1.267
Thamnophilidae	Thamnophilus_nigriceps	1.246	1.365	0.795	1.093	1.36
Thamnophilidae	Thamnophilus_palliatus	1.222	1.358	0.783	0.993	1.431
Thamnophilidae	Thamnophilus_punctatus	1.216	1.275	0.795	0.929	1.301
Thamnophilidae	Thamnophilus_ruficapillus	1.193	1.329	0.750	1.007	1.352
Thamnophilidae	Thamnophilus_schistaceus	1.231	1.308	0.789	0.930	1.301
Thamnophilidae	Thamnophilus_stictocephalus	1.232	1.324	0.782	1.062	1.312
Thamnophilidae	Thamnophilus_tenuepunctatus	1.205	1.330	0.768	0.994	1.352
Thamnophilidae	Thamnophilus_torquatus	1.186	1.271	0.744	0.885	1.279
Thamnophilidae	Thamnophilus_unicolor	1.222	1.309	0.793	1.048	1.342
Thamnophilidae	Thamnophilus_zarumae	1.161	1.345	0.752	0.996	1.342
Thamnophilidae	Willisornis_poecilinotus	1.201	1.294	0.673	0.926	1.265
Tyrannidae	Anairetes_parulus	0.968	1.070	0.417	0.617	0.778
Tyrannidae	Arundinicola_leucocephala	1.128	1.258	0.665	0.922	1.14
Tyrannidae	Attila_spadiceus	1.302	1.402	0.820	1.121	1.576
Tyrannidae	Camptostoma_obsoletum	0.916	1.073	0.512	0.840	0.903
Tyrannidae	Capsiempis_flaveola	0.968	1.069	0.568	0.809	0.903
Tyrannidae	Casiornis_rufus	1.177	1.300	0.715	1.038	1.389
Tyrannidae	Cnemotriccus_fuscatus	1.069	1.209	0.636	0.887	1.076
Tyrannidae	Cnipodectes_subbrunneus	1.160	1.278	0.729	0.974	1.365
Tyrannidae	Colonia_colonus	0.953	1.137	0.580	1.010	1.217
Tyrannidae	Corythopis_delalandi	1.150	1.237	0.576	0.928	1.217

Tyrannidae	Deltarhynchus_flammulatus	1.155	1.262	0.659	0.975	1.236
Tyrannidae	Empidonomus_varius	1.139	1.294	0.756	1.061	1.398
Tyrannidae	Euscarthmus_meloryphus	1.003	1.119	0.509	0.822	0.845
Tyrannidae	Fluvicola_albiventer	1.102	1.240	0.572	0.881	1.064
Tyrannidae	Gubernetes_yetapa	1.304	1.419	0.947	1.098	1.562
Tyrannidae	Hemitriccus_diops	1.021	1.152	0.552	0.878	1.061
Tyrannidae	Hemitriccus_margaritaceiventer	1.113	1.171	0.550	0.815	0.929
Tyrannidae	Hirundinea_ferruginea	1.206	1.335	0.701	1.037	1.322
Tyrannidae	Hymenops_perspicillatus	1.158	1.272	0.660	0.887	1.36
Tyrannidae	Inezia_inornata	0.929	1.066	0.439	0.703	0.76
Tyrannidae	Lathrotriccus_euleri	1.049	1.194	0.602	0.897	1.041
Tyrannidae	Legatus_leucophaius	1.041	1.171	0.722	0.960	1.389
Tyrannidae	Leptopogon_amaurocephalus	1.075	1.192	0.606	0.875	1.079
Tyrannidae	Lophotriccus_pileatus	1.026	1.095	0.500	0.764	0.875
Tyrannidae	Machetornis_rixosus	1.248	1.351	0.720	0.953	1.471
Tyrannidae	Mecocerculus_leucophrys	0.981	1.085	0.481	0.720	1.017
Tyrannidae	Mecocerculus_poecilocercus	0.929	1.047	0.457	0.701	1.021
Tyrannidae	Megarynchus_pitangua	1.462	1.542	1.054	1.237	1.82
Tyrannidae	Mionectes_rufiventris	1.056	1.191	0.587	0.904	1.146
Tyrannidae	Mitrephanes_phaeocercus	0.986	1.099	0.506	0.771	0.929
Tyrannidae	Muscigralla_brevicauda	1.090	1.203	0.582	0.814	1.1
Tyrannidae	Muscisaxicola_maculirostris	1.094	1.199	0.535	0.749	1.152
Tyrannidae	Myiarchus_tyrannulus	1.238	1.339	0.816	1.026	1.641
Tyrannidae	Myiodynastes_maculatus	1.298	1.444	0.933	1.149	1.643
Tyrannidae	Myiopagis_caniceps	0.982	1.100	0.566	0.870	1.021
Tyrannidae	Myiopagis_viridicata	0.989	1.118	0.572	0.883	1.114
Tyrannidae	Myiophobus_cryptoxanthus	1.033	1.113	0.551	0.846	0.991
Tyrannidae	Myiophobus_fasciatus	1.031	1.178	0.575	0.857	1.092
Tyrannidae	Myiophobus_phoenicomitra	1.019	1.127	0.559	0.849	1.041
Tyrannidae	Myiophobus_pulcher	0.976	1.054	0.445	0.804	0.978
Tyrannidae	Myiornis_auricularis	0.967	1.085	0.475	0.758	0.699
Tyrannidae	Myiotheretes_fumigatus	1.278	1.403	0.761	1.004	1.524
Tyrannidae	Myiotriccus_ornatus	1.011	1.120	0.675	0.858	1.097
Tyrannidae	Myiozetetes_similis	1.091	1.226	0.723	0.972	1.237
Tyrannidae	Neopipo_cinnamomea	0.916	1.035	0.453	0.733	0.845
Tyrannidae	Ochthoeca_cinnamomeiventris	0.947	1.198	0.559	0.862	1.076
Tyrannidae	Ochthoeca_diadema	0.978	1.128	0.525	0.805	1.061
Tyrannidae	Ornithion_brunneicapillus	0.919	1.035	0.533	0.709	0.875
Tyrannidae	Phaeomyias_murina	0.982	1.084	0.531	0.801	0.903
Tyrannidae	Phylloscartes_ventralis	1.010	1.107	0.473	0.711	0.954
Tyrannidae	Phyllomyias_fasciatus	0.868	1.013	0.528	0.754	1.013
Tyrannidae	Phyllomyias_griseiceps	0.885	1.027	0.501	0.769	0.857
Tyrannidae	Phyllomyias_uropygialis	0.910	1.014	0.451	0.720	0.954

Tyrannidae	Pitangus_sulphuratus	1.395	1.486	0.965	1.117	1.785
Tyrannidae	Platyrinchus_mystaceus	0.972	1.149	0.526	0.934	1.002
Tyrannidae	Polystictus_pectoralis	0.960	1.067	0.466	0.742	0.845
Tyrannidae	Pseudotriccus_ruficeps	1.048	1.118	0.455	0.707	0.903
Tyrannidae	Pyrocephalus_rubinus	1.040	1.205	0.603	0.884	1.097
Tyrannidae	Pyrrhomyias_cinnamomea	0.942	1.078	0.493	0.793	0.996
Tyrannidae	Ramphotrigon_megacephalum	1.064	1.203	0.672	0.941	1.146
Tyrannidae	Ramphotrigon_ruficauda	1.131	1.283	0.707	1.030	1.296
Tyrannidae	Rhytipterna_simplex	1.237	1.374	0.809	0.997	1.55
Tyrannidae	Sayornis_nigricans	1.117	1.278	0.613	0.913	1.255
Tyrannidae	Serpophaga_munda	0.900	1.017	0.540	0.668	0.903
Tyrannidae	Sirystes_sibilator	1.196	1.324	0.824	1.064	1.502
Tyrannidae	Stigmatura_budytoides	1.013	1.129	0.506	0.807	1.035
Tyrannidae	Sublegatus_modestus	0.900	1.063	0.515	0.836	1.051
Tyrannidae	Suiriri_suiriri	1.015	1.157	0.641	0.877	1.138
Tyrannidae	Tachuris_rubrigastra	1.012	1.098	0.418	0.606	0.86
Tyrannidae	Terenotriccus_erythrurus	0.917	1.032	0.439	0.775	0.845
Tyrannidae	Todirostrum_cinereum	1.102	1.197	0.536	0.766	0.643
Tyrannidae	Tolmomyias_flaviventris	0.968	1.142	0.590	0.918	1.053
Tyrannidae	Tumbezia_salvini	1.089	1.201	0.593	0.791	1.086
Tyrannidae	Tyrannus_savana	1.171	1.290	0.746	0.980	1.498
Pipridae	Antilophia_galeata	0.890	1.097	0.629	0.930	1.343
Pipridae	Chiroxiphia_caudata	0.972	1.116	0.686	0.971	1.408
Pipridae	Chiroxiphia_linearis	0.836	1.170	0.581	0.924	1.242
Pipridae	Corapipo_gutturalis	0.813	1.088	0.519	0.851	0.914
Pipridae	Corapipo_leucorrhoa	0.853	1.076	0.533	0.822	1.068
Pipridae	Ilicura_militaris	0.716	1.080	0.477	0.845	1.104
Pipridae	Lepidothrix_coronata	0.928	1.075	0.561	0.848	0.929
Pipridae	Lepidothrix_iris	0.975	1.086	0.642	0.820	0.937
Pipridae	Lepidothrix_isidorei	0.756	1.013	0.421	0.797	0.892
Pipridae	Lepidothrix_nattereri	0.928	1.052	0.582	0.821	0.964
Pipridae	Lepidothrix_serena	0.924	1.080	0.609	0.885	1.041
Pipridae	Machaeropterus_deliciosus	0.906	1.119	0.493	0.941	1.104
Pipridae	Machaeropterus_pyrocephalus	0.905	0.999	0.557	0.877	0.991
Pipridae	Manacus_aurantiacus	0.940	1.172	0.585	0.989	1.19
Pipridae	Manacus_candei	0.974	1.232	0.599	0.967	1.298
Pipridae	Manacus_manacus	0.974	1.084	0.624	0.834	1.227
Pipridae	Manacus_vitellinus	0.964	1.212	0.602	0.963	1.26
Pipridae	Masius_chrysopterus	0.782	1.131	0.559	0.888	1.063
Pipridae	Neopelma_chrysocephalum	1.009	1.235	0.610	0.989	1.19
Pipridae	Neopelma_pallescens	1.021	1.250	0.642	1.015	1.26
Pipridae	Neopelma_sulphureiventer	1.060	1.137	0.628	0.887	1.236
Pipridae	Pipra_aureola	0.978	1.074	0.647	0.878	1.211

Pipridae	Pipra_cornuta	0.944	1.234	0.629	1.035	1.403
Pipridae	Pipra_erythrocephala	0.915	1.102	0.615	0.912	1.107
Pipridae	Pipra_fasciicauda	0.980	1.123	0.618	0.859	1.183
Pipridae	Pipra_filicauda	0.947	1.200	0.595	0.996	1.188
Pipridae	Pipra_mentalis	0.899	1.157	0.574	0.963	1.176
Pipridae	Dixiphia_pipra	1.070	0.634	0.849	0.849	1.146
Pipridae	Pipra_rubrocapilla	0.917	1.108	0.576	0.917	1.092
Pipridae	Tyranneutes_stolzmanni	0.970	1.024	0.566	0.835	0.927
Pipridae	Tyranneutes_virescens	0.926	1.128	0.488	0.856	0.857
Pipridae	Xenopipo_atronitens	1.044	1.104	0.652	0.929	1.183
Parulidae	Basileuterus_belli	1.024	1.131	0.572	0.779	1
Parulidae	Basileuterus_culicivorus	1.021	1.127	0.560	0.814	1.031
Parulidae	Basileuterus_lachrymosa	1.096	1.208	0.618	0.822	1.19
Parulidae	Basileuterus_melanogenys	1.039	1.133	0.587	0.783	1.072
Parulidae	Basileuterus_rufifrons	1.031	1.131	0.600	0.827	1.061
Parulidae	Basileuterus_tristriatus	1.025	1.140	0.635	0.833	1.064
Parulidae	Cardellina_canadensis	0.959	1.147	0.542	0.731	1.041
Parulidae	Cardellina_pusilla	0.952	1.053	0.451	0.701	0.9
Parulidae	Cardellina_rubrifrons	0.950	1.064	0.580	0.766	0.987
Parulidae	Geothlypis_aequinoctialis	1.096	1.190	0.645	0.803	1.117
Parulidae	Geothlypis_agilis	1.058	1.170	0.573	0.763	1.273
Parulidae	Geothlypis_flavovelata	1.071	1.187	0.544	0.748	1.035
Parulidae	Geothlypis_formosus	1.073	1.185	0.581	0.813	1.204
Parulidae	Geothlypis_nelsoni	1.047	1.130	0.572	0.775	1.039
Parulidae	Geothlypis_philadelphia	1.032	1.159	0.568	0.786	1.138
Parulidae	Geothlypis_poliocephala	1.078	1.176	0.688	0.872	1.17
Parulidae	Geothlypis_rostrata	1.177	1.260	0.653	0.856	1.21
Parulidae	Geothlypis_semiflava	1.087	1.203	0.627	0.770	1.23
Parulidae	Geothlypis_tolmiei	1.013	1.136	0.542	0.757	1.025
Parulidae	Geothlypis_trichas	1.036	1.149	0.531	0.755	1.063
Parulidae	Helmitheros_vermivorus	1.143	1.200	0.684	0.778	1.164
Parulidae	Limnothlypis_swainsonii	1.170	1.243	0.676	0.746	1.239
Parulidae	Mniotilta_varia	1.079	1.170	0.510	0.728	1.079
Parulidae	Myioborus_brunniceps	0.993	1.096	0.521	0.734	0.971
Parulidae	Myioborus_melanocephalus	0.943	1.129	0.535	0.794	1.061
Parulidae	Myioborus_miniatus	0.964	1.092	0.526	0.748	1.021
Parulidae	Myioborus_pictus	0.967	1.086	0.543	0.742	0.94
Parulidae	Myioborus_torquatus	1.003	1.096	0.561	0.785	1.021
Parulidae	Myiothlypis_bivittatus	1.030	1.151	0.619	0.838	1.164
Parulidae	Myiothlypis_chrysogaster	0.914	1.154	0.556	0.866	1.045
Parulidae	Myiothlypis_coronatus	1.060	1.159	0.643	0.814	1.217
Parulidae	Myiothlypis_flaveolus	1.045	1.196	0.583	0.851	0
Parulidae	Myiothlypis_fraseri	1.041	1.201	0.580	0.881	1.064

Parulidae	Myiothlypis_fulvicauda	1.114	1.190	0.631	0.840	1.173
Parulidae	Myiothlypis_leucoblepharus	1.048	1.152	0.578	0.747	1.243
Parulidae	Myiothlypis_luteoviridis	1.010	1.169	0.593	0.837	1.217
Parulidae	Myiothlypis_nigrocristatus	1.037	1.192	0.556	0.820	1.189
Parulidae	Myiothlypis_rivularis	1.036	1.214	0.568	0.838	1.146
Parulidae	Myiothlypis_signatus	1.004	1.188	0.580	0.810	1.106
Parulidae	Oreothlypis_celata	0.981	1.109	0.511	0.660	0.975
Parulidae	Oreothlypis_crissalis	1.022	1.152	0.547	0.626	0.989
Parulidae	Oreothlypis_gutturalis	1.046	1.157	0.561	0.732	0.978
Parulidae	Oreothlypis_luciae	0.903	1.049	0.456	0.585	0.813
Parulidae	Oreothlypis_peregrina	1.000	1.104	0.513	0.631	1.09
Parulidae	Oreothlypis_ruficapilla	0.966	1.105	0.489	0.640	1.013
Parulidae	Oreothlypis_superciliosa	1.003	1.120	0.533	0.732	1
Parulidae	Oreothlypis_virginiae	0.961	1.081	0.502	0.615	0.916
Parulidae	Parkesia_motacilla	1.151	1.245	0.629	0.805	1.33
Parulidae	Parkesia_noveboracensis	1.101	1.191	0.577	0.769	1.281
Parulidae	Protonotaria_citrea	1.154	1.244	0.631	0.887	1.225
Parulidae	Seiurus_aurocapilla	1.083	1.199	0.626	0.798	1.316
Parulidae	Setophaga_americana	0.991	1.073	0.518	0.692	0.937
Parulidae	Setophaga_caerulescens	1.004	1.100	0.494	0.736	1.017
Parulidae	Setophaga_castanea	1.029	1.138	0.582	0.779	1.102
Parulidae	Setophaga_cerulea	1.009	1.122	0.549	0.738	0.971
Parulidae	Setophaga_chrysoparia	0.991	1.151	0.591	0.808	1.035
Parulidae	Setophaga_citrina	0.994	1.152	0.541	0.795	1.041
Parulidae	Setophaga_coronata	0.994	1.088	0.542	0.759	1.161
Parulidae	Setophaga_discolor	0.995	1.104	0.489	0.705	0.916
Parulidae	Setophaga_fusca	0.997	1.099	0.531	0.735	0.987
Parulidae	Setophaga_graciae	1.010	1.109	0.507	0.703	0.919
Parulidae	Setophaga_kirtlandii	1.012	1.185	0.606	0.806	1.149
Parulidae	Setophaga_magnolia	0.983	1.081	0.536	0.752	0.982
Parulidae	Setophaga_nigrescens	0.982	1.091	0.527	0.715	0.911
Parulidae	Setophaga_occidentalis	0.985	1.106	0.532	0.718	1.011
Parulidae	Setophaga_palmarum	1.018	1.123	0.513	0.716	0.998
Parulidae	Setophaga_pensylvanica	0.995	1.099	0.575	0.755	1.013
Parulidae	Setophaga_petechia	1.005	1.115	0.521	0.721	1.068
Parulidae	Setophaga_pitiayumi	0.982	1.081	0.513	0.715	0.816
Parulidae	Setophaga_ruticilla	0.975	1.107	0.504	0.758	0.966
Parulidae	Setophaga_striata	1.006	1.126	0.540	0.743	1.185
Parulidae	Setophaga_tigrina	1.000	1.085	0.525	0.732	1.116
Parulidae	Setophaga_townsendi	0.988	1.097	0.540	0.734	0.954
Parulidae	Setophaga_virens	0.985	1.092	0.552	0.730	0.971
Turdidae	Catharus_aurantiirostris	1.137	1.295	0.651	0.925	1.423
Turdidae	Catharus_bicknelli	1.140	1.278	0.623	0.923	1.47

Turdidae	Catharus_dryas	1.186	1.322	0.680	0.884	1.602
Turdidae	Catharus_frantzii	1.162	1.292	0.646	0.888	1.477
Turdidae	Catharus_fuscater	1.164	0.964	0.669	0.888	1.562
Turdidae	Catharus_fuscescens	1.100	1.308	0.657	0.984	1.531
Turdidae	Catharus_gracilirostris	1.093	1.234	0.584	0.838	1.322
Turdidae	Catharus_guttatus	1.150	1.290	0.620	0.930	1.439
Turdidae	Catharus_mexicanus	1.149	1.312	0.661	0.931	1.477
Turdidae	Catharus_minimus	1.136	1.297	0.639	0.909	1.58
Turdidae	Catharus_occidentalis	1.138	1.266	0.613	0.900	1.415
Turdidae	Catharus_ustulatus	1.113	1.272	0.633	0.964	1.544
Turdidae	Cichlopsis	1.172	1.269	0.706	0.927	1.724
Turdidae	Entomodestes_coracinus	1.185	1.314	0.694	0.967	1.748
Turdidae	Entomodestes_leucotis	1.182	1.337	0.689	0.968	1.763
Turdidae	Hylocichla	1.219	1.381	0.730	1.025	1.748
Turdidae	Myadestes	1.065	1.234	0.646	0.928	1.464
Turdidae	Sialia_currucoides	1.117	1.247	0.628	0.902	1.477
Turdidae	Sialia_mexicanus	1.085	1.224	0.625	0.904	1.422
Turdidae	Sialia_sialis	1.075	1.225	0.648	0.930	1.462
Turdidae	Turdus_chiguanco	1.397	1.476	0.884	1.018	2.029
Turdidae	Platycichla	1.248	1.375	0.784	0.992	1.803
Turdidae	Turdus_fuscater	1.381	1.486	0.929	1.042	2.18
Turdidae	Turdus_grayi	1.293	1.423	0.813	1.020	1.878
Turdidae	Turdus_migratorius	1.285	1.403	0.825	1.010	1.884
Turdidae	Turdus_rufiventris	1.287	1.391	0.835	0.937	1.875

Table S2. PGLS models. Model 1: FB ~ PCbeak + logMASS; Model 2: FB ~ PCbeak; Model 3: FB ~ PCbeak * logMASS; Model 4: SR ~ PCbeak + logMASS; Model 5: SR ~ PCbeak; Model 6: SR ~ PCbeak * logMASS.

Family	**Model**	**Variable**	**Value**	**SE**	**t-value**	**p-value**
Thamnophilidae	mod1 (AIC = 1750.698)	PC1beak	-668.929	415.458	-1.610	0.110
		logMASS	-575.511	366.307	-1.571	0.119
	mod2 (AIC = 1764.801)	PC1beak	-970.896	370.778	-2.619	**0.010**
	mod3 (AIC = 1731.366)	PC1beak	-6802.967	3049.525	-2.231	**0.028**
		logMASS	-817.405	380.325	-2.149	**0.034**
		PC1beak:logMASS	4520.967	2227.220	2.030	**0.045**
	mod4 (AIC = 755.651)	PC1beak	-10.752	4.327	-2.485	**0.015**
		logMASS	7.218	3.815	1.892	0.061
	mod5 (AIC = 761.7168)	PC1beak	-6.964	3.881	-1.794	0.076
	mod6 (AIC= 739.114)	PC1beak	90.130	30.845	2.922	**0.004**
		logMASS	11.197	3.847	2.911	**0.004**
		PC1beak:logMASS	-74.353	22.528	-3.301	**0.001**

Tyrannidae	mod1 (AIC = 1240.065)	pc1beak	-2766.160	1719.597	-1.609	0.112
		logMASS	-404.732	1728.860	-0.234	0.816
	mod2 (AIC = 1254.861)	pc1beak	-3117.720	832.229	-3.746	**0.000**
	mod3 (AIC = 1224.864)	PC1beak	-2915.089	3674.020	-0.793	0.430
		logMASS	-401.442	1742.610	-0.230	0.819
		PC1beak:logMASS	99.878	2173.052	0.046	0.964
	mod4 (AIC = 529.224)	PC1beak	-4.575	11.518	-0.397	0.692
		logMASS	-3.639	11.580	-0.314	0.754
	mod5 (AIC = 534.054)	PC1beak	-7.736	5.576	-1.387	0.170
	mod6 (AIC = 523.626)	PC1beak	-18.472	24.537	-0.753	0.454
		logMASS	-3.332	11.638	-0.286	0.776
		PC1beak:logMASS	9.320	14.513	0.642	0.523
Pipridae	mod1 (AIC = 506.879)	PC1beak	-846.330	2563.522	-0.330	0.744
		PC2beak	-6040.487	4390.207	-1.376	0.180

	logMASS	-3382.075	3784.205	-0.894	0.379
mod2 (AIC = 523.992)	PC1beak	42.768	2354.413	0.018	0.986
	PC2beak	-3759.153	3559.452	-1.056	0.300
mod3 (AIC = 464.994)	PC1beak	-32939.120	36128.710	-0.912	0.370
	PC2beak	19734.280	22829.500	0.864	0.395
	logMASS	-4548.350	4081.330	-1.114	0.275
	PC1beak:logMASS	27146.290	30569.390	0.888	0.383
	PC2beak:logMASS	-26060.020	22490.070	-1.159	0.257
mod4 (AIC = 205.076)	PC1beak	6.738	11.702	0.576	0.569
	PC2beak	12.051	20.041	0.601	0.553
	logMASS	19.259	17.275	1.115	0.274
mod5 (AIC = 211.854)	PC1beak	1.675	10.830	0.155	0.878
	PC2beak	-0.940	16.374	-0.057	0.955
mod6 (AIC = 185.587)	PC1beak	-131.872	167.608	-0.787	0.439
	PC2beak	64.703	105.910	0.611	0.547

		logMASS	18.238	18.934	0.963	0.344
		PC1beak:logMASS	117.481	141.817	0.828	0.415
		PC2beak:logMASS	-53.938	104.336	-0.517	0.610
Parulidae	mod1 (AIC = 664.828)	PC1beak	15.729	43.589	0.361	0.719
		logMASS	-10.452	37.006	-0.282	0.778
	mod2 (AIC = 671.962)	PC1beak	23.447	33.740	0.695	0.489
	mod3 (AIC = 648.139)	**PC1beak**	-498.138	202.713	-2.457	**0.017**
		logMASS	-36.393	36.982	-0.984	0.329
		PC1beak:logMASS	459.554	177.366	2.591	**0.012**
	mod4 (AIC = 672.557	PC1beak	96.357	46.027	2.093	**0.040**
		logMASS	22.896	39.076	0.586	0.560
	mod5 (AIC = 680.066)	PC1beak	79.448	35.693	2.226	**0.029**
	mod6 (AIC = 661.585)	PC1beak	263.657	223.147	1.182	0.241
		logMASS	31.342	40.710	0.770	0.444

		PC1beak:logMASS	-149.617	195.245	-0.766	0.446
Turdidae	mod1 (AIC = 428.114)	PC1beak	2752.499	8691.206	0.317	0.754
		logMASS	-2287.250	5799.254	-0.394	0.697
	mod2 (AIC = 445.424)	PC1beak	210.723	5727.808	0.037	0.971
	mod3 (AIC = 409.029)	PC1beak	9825.996	26719.626	0.368	0.717
		logMASS	-1790.769	6177.715	-0.290	0.775
		PC1beak:logMASS	-4117.457	14671.248	-0.281	0.782
	mod4 (AIC = 89.172)	PC1beak	0.352	5.484	0.064	0.949
		logMASS	-0.147	3.659	-0.040	0.968
	mod5 (AIC = 91.585)	PC1beak	0.189	3.602	0.052	0.959
	mod6 (AIC = 84.332)	PC1beak	-11.607	16.671	-0.696	0.494
		logMASS	-0.986	3.854	-0.256	0.801
		PC1beak:logMASS	6.961	9.154	0.760	0.455