UNIVERSIDAD DE COSTA RICA
SISTEMA DE ESTUDIOS DE POSTGRADO
PROGRAMA REGIONAL DE MAESTRIA EN BIOLOGÍA.

INFORMACIÓN SOCIAL EN EL CANTO DE *PHAENOSTICTUS MCLEANNANI*,
UN AVE SEGUIDORA DE HORMIGAS ARRIERAS.

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado
en Biología para optar por el grado de Magister Scientiae en Biología.
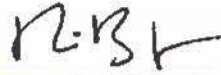

YI-MEN GERARDO ARAYA AJOY

Ciudad Universitaria Rodrigo Facio, Costa Rica

2009

DEDICATORIA Y AGRADECIMIENTOS

"Esta tesis fue aceptada por la Comisión del Programa de Estudios de Posgrado en Biología de la Universidad de Costa Rica, como requisito parcial para optar al grado de Magister Scientiae en Biología."

Dr. Ramiro Barrantes Mesén

Representante de la Decana

Sistema de Estudios de Posgrado

Dr. Johel Chaves Campos

Director de Tesis

Dr. Gilbert Barrantes Montero

Asesor

Dr. Edgardo Arévalo Hernández

Asesor

M.Sc Federico Bolaños Vives

Representante del Director

Programa de Estudios de Posgrado en Biología

Yi-men Araya Ajoy

Candidato

iii

TABLA DE CONTENIDOS

Articulo científico 1. Las notas con frecuencias altas durante las competencias vocales de *Phaenostictus mcleannani* (Thanmophilidae) se relacionan con la diversidad genética del emisor.

Artículo científico 2. La variación en las vocalizaciones de contacto de *Phanesotictus mcleannani* permite reconocer vecinos territoriales en sus relaciones de
tolerancia reciproca.

LISTA DE CUADROS

LISTA DE FIGURAS

PREFACIO

Esta tesis fue escrita de acuerdo al formato de escritura en forma de artículos científicos aprobado para el Programa de Posgrado en Biología. El trabajo contiene este prefacio que posee una introducción general al tema y dos capítulos que corresponden a artículos científicos que están en proceso de publicación. Cada capítulo esta escrito para ser leído independientemente y por esta razón fue necesario repetir información a través de los capítulos.

La comunicación biológica puede ocurrir entre células, órganos o individuos (Scott-Phillips 2008) y posee tres componentes: el emisor, el receptor y la señal. Las señales reducen la incertidumbre de las interacciones entre emisor y receptor. Los emisores usan las señales para modificar el comportamiento del receptor de una forma predecible, mientras que las respuestas de los receptores han evolucionado para explotar la información de la señal (Hurd & Enquist 2005). Si las señales brindan información falsa o deshonesta el receptor eventualmente dejará de usar la señal. Las señales que no producen una respuesta por parte del receptor dejaran de ser emitidas, por lo tanto las señales deberán brindar información en promedio honesta para ser evolutivamente estables (Smith & Harper 1995). Estudiar como la interacción entre emisor y receptor ha moldeado la estructura de la señal es importante para entender como la información es transferida.

Durante la comunicación el emisor y el receptor pueden tener los mismos o diferentes intereses. Cuando los intereses de emisores y receptores coinciden, el emisor no tiene ninguna motivación para engañar al receptor. Pero cuando existe un conflicto de intereses entre emisor y receptor, puede existir una ventaja para el emisor en engañar al receptor (Hurd & Enquist 2005). Las vocalizaciones de las aves han sido utilizadas como modelos para estudiar las señales en ambos tipos de contextos, pero a veces no existe un límite claro entre los dos escenarios (Slater 2003). Los llamados de los polluelos por alimento son señales en contextos donde no existe conflicto entre los intereses del emisor y receptor. Los padres se benefician alimentando a los polluelos y para los polluelos obviamente es bueno que los alimenten (Johnstone 1999; Leonard & Horn 2001). Lo contrario ocurre cuando los

existe conflicto entre los intereses del emisor y receptor. Los padres se benefician alimentando a los polluelos y para los polluelos obviamente es bueno que los alimenten (Johnstone 1999; Leonard & Horn 2001). Lo contrario ocurre cuando los machos cantan para atraer posibles parejas. Los machos de buena "calidad" producen cantos atractivos para las hembras. Las hembras escogen a los machos de acuerdo con la atractividad del canto (Catchpole 1987), pero que evita que un macho de mala calidad produzca cantos atractivos y las hembras sean engañadas. La explicación mas aceptadas es que los machos de baja calidad no pueden producir cantos atractivos por que son difíciles o costosos de producir (Gil & Gahr 2002).

Un caso donde los intereses del emisor y el receptor no coinciden pero tampoco son completamente contradictorios es durante las interacciones de vecinos territoriales. Los individuos pueden reconocer a sus vecinos territoriales por su canto, lo que reduce los costos asociados a la defensa territorial. Los individuos "extraños o no vecinos" son una mayor amenaza para el dueño del territorio, ya que los vecinos territoriales poseen un territorio y una pareja, mientras que los extraños probablemente no (Temeles 1994). La habilidad de reconocer a los vecinos territoriales por el canto permite que el dueño del territorio pueda enfocar sus esfuerzos agresivos hacia individuos que representan una verdadera amenaza para ellos (Temeles 1994). Por su parte los individuos que posee señales que brinden información acerca de su identidad recibirán menores niveles de agresión por parte de sus vecinos territoriales (Tibbetts & Dale 2007).

La variación entre individuos es un requisito para que las señales brinden información acerca de la calidad u identidad del emisor, independientemente de que exista o no conflicto de intereses entre receptor y emisor (Falls 1982). La variación entre individuos puede deberse a modificaciones en la estructura de las vocalizaciones debido al proceso de aprendizaje o a diferencias neurológicas y morfológicas (Suthers et al. 1999), pero también puede estar relacionada a la condición física del emisor (Gil & Gahr 2002). La contribución relativa de estos factores determina la estructura de la vocalización y cual es la información que puede ser extraída de la señal (Podos et al., 2004). El canto en aves es una señal compleja

debido a que posee múltiples componentes y la variación individual puede ocurrir en cualquiera de sus componentes. Señales compuestas de varios componentes pueden servir para reforzar la información de un aspecto del emisor o los diferentes componentes pueden brindar información de diferentes aspectos del emisor (Johnstone 1996).

Los diferentes componentes o características del canto pueden agruparse en cuatro categorías: características espectrales, características temporales, características relacionadas con la amplitud y características asociadas a la complejidad del canto. Para la producción exitosa del canto es necesaria la coordinación neuromotora de los cuatro aspectos (Suthers et al. 1999). Las características espectrales son determinadas por la siringe donde la tensión de los labios son los que determinan la frecuencia fundamental de las notas (Suthers et al. 1999). Las características temporales del canto están determinadas por el ritmo respiratorio del individuo mientras canta (Goller & Suthers 1996). Las características relacionadas con la amplitud de la vocalización son determinadas por los músculos que contraen los sacos aéreos; estas características son las menos estudiadas por las dificultades para cuantificarlas (Forstmeier et al. 2002; Janicke et al. 2008), por el contrario, las características que describen la complejidad del canto son las mas estudiadas, especialmente en los repertorios aprendidos de los passeriformes (Hasselquist et al. 1996; Nowicki & Searcy 2004; Pfaff et al. 2007). Los cantos aprendidos de algunos passeriformes han sido modelo en el estudio de las bases neurológicas del aprendizaje (Amin et al. 2004; Mooney et al. 2002; Volman 1993) y la comunicación animal en general (Slater 2003).

Como se mencionó anteriormente la mayoría de los estudios del canto en aves se han realizado en especies que aprende el canto durante el desarrollo y también durante las interacciones vocales con otros individuos inclusive cuando ya son adultos. Se ha demostrado que algunas en algunas especies los individuos reconocen a sus vecinos territoriales usando elementos que comparten con ellos. Estos elementos son aprendidos durante sus interacciones vocales (Beecher et al. 2000; Price 1999). El mismo mecanismo es utilizado por algunas especies que viven

en grupos estables, para reconocer a sus compañeros de grupo y también parientes (Hopp et al. 2001; Price 1999). También se ha estudiado cómo el tamaño y la complejidad de los cantos aprendidos se relacionan con la calidad del emisor (Gil & Gahr 2002; Searcy 1992). Otro contexto bien estudiado pero no necesariamente relacionado con el aprendizaje del canto, es en los animales que la habilidad como competidor se relaciona con el tamaño, y las frecuencias bajas en las vocalizaciones durante interacciones agresivas indican su tamaño. Se ha demostrado que en estas situaciones los rivales y parejas potenciales usan las frecuencias bajas para evaluar la calidad del emisor (Mahler & Tubaro 2001; Price 1999; Ryan & Brenowitz 1985).

A diferencia de las aves que aprenden su canto, la comunicación acústica en especies que no aprenden el canto ha sido pobremente estudiada. Se cree que las aves en el suborden de passeriformes suboscines no necesitan aprender el canto durante su desarrollo, tampoco se tiene evidencia que puedan aprender variaciones del canto cuando son adultos(Kroodsma 1984; Kroodsma & Konishi 1991). El aprendizaje es importante para la producción del canto en todos los oscines estudiados lo que sugiere que la capacidad de aprender las vocalizaciones evoluciono después de que los dos subordenes de passeriformes divergieron (Kroodsma & Konishi 1991). A pesar de su dominancia en avifaunas tropicales y su potencial como modelos para el estudio de vocalizaciones no aprendidas se han realizado pocos estudios en la comunicación acústica de los suboscines. Una revisión de literatura publicada en 1995 reveló que de 418 estudios en bioacústica solo 4 fueron sobre suboscines. Yo realice una revisión de literatura posterior y encontré solo 9 artículos adicionales acerca de subocines desde 1995 (Bard et al. 2002; Fedy & Stutchbury 2005; Lovell & Lein 2004; Lovell & Lein 2005; Morton & Derrickson 1996; Seddon 2005; Seddon et al. 2008; Seddon & Tobias 2006; Seddon & Tobias 2007)

Las aves de la familia thamnophilidae comúnmente llamados "hormigueros" están surgiendo como modelos en bioacústica dentro de los suboscines, debido a estudios comparativos entre varias especies realizados por Nathalie Seddon (Seddon 2005; Seddon et al. 2008; Seddon & Tobias 2006; Seddon & Tobias 2007). Ella encontró que la estructura de las vocalizaciones de aves se ha moldeado para

adaptarse a diferentes ambientes acústicos, también encontró que algunas características de las vocalizaciones son un subproducto de adaptaciones morfológicas (Seddon 2005; Seddon et al. 2008). Otra de sus conclusiones fue que la presión de especies hermanas simpátricas ha moldeado las vocalizaciones para prevenir la hibridización. (Seddon & Tobias 2007). Al nivel de especie en los aves de la familia thamnophilidae solo se han realizado tres estudios acerca de la función del canto; uno acerca de la función del canto de las hembras de *Cercomacra tyrannina* (Fedy & Stutchbury 2005), otro acerca la función de los duetos en *Hypocnemis cantator* (Seddon & Tobias 2006) y un estudio acerca de discriminación acústica entre vecinos territoriales en *Hylophylax naveoides* (Bard et al. 2002). Los hormigueros son buenos modelos para estudiar la comunicación animal ya que muchas especies poseen plumajes poco diferenciados y viven en el bosque con condiciones pobres de luz, lo que aumenta su dependencia en señales acústicas para reconocimiento individual y selección sexual. Es mas, algunas especies se alimentan exclusivamente de los artrópodos que tratan de escapar de las hormigas arrieras (*Eciton burchelli* y *Labidus predator*) y para poder explotar este recurso óptimamente forman grupos estables diariamente durante el forrajeo (Willis and Oniki 1978). Esto brinda la oportunidad de estudiar como se utilizan las vocalizaciones no aprendidas para manejar relaciones sociales complejas, esto adquiere mas importancia al no haber estudios en la función de las vocalizaciones en ningún suboscines social.

Yo estudie reconocimiento individual y selección intra sexual en el canto del suboscine social *Phaenostictus mcleannani*, (Thmanophilidae). Este ave es el mas grande, menos común y mas dependiente de los seguidores de hormigas arrieras en los bosques tropicales de Costa Rica y Panamá (Stiles & Skutch 1989; Willis & Oniki 1978). Willis (1973) estudio la historia natural de esta especie desde 1960 hasta 1971 en la isla de Barro Colorado e identificó 16 diferentes tipos de vocalizaciones. Yo enfoque mi investigación en una vocalización que producen tanto machos como hembras de *P. mcleannani* llamada "loud song" (sensu Willis 1973). Esta vocalización consiste en elementos que aumentan en tono, tasa de repetición y

amplitud, que luego descienden en tono, repetición y amplitud hacia el final de la vocalización, terminando en unos elementos de sonido áspero y numero variable (Fig 1).

Phaenostictus mcleannani forma parejas que son socialmente monógamas y los "loud songs" son usados para contactar a la pareja cuando llegan o se van de los enjambres de hormigas. Estas vocalizaciones también son producidas durante interacciones agresivas entre individuos, que ocasionalmente terminan cuando el ave dominante persigue agresivamente al otro (Willis 1973). Las características de las vocalizaciones varían entre contextos; cuando es utilizado para contactar a la pareja las vocalizaciones son cortas en duración, mas bajas en tono y amplitud. Mientras que durante las interacciones agresivas es cuando las vocalizaciones tienen mayor amplitud, tono y duración (obs. per.).

El objetivo general de mi investigación es estudiar la información que puede ser extraída de las características temporales y espectrales de las vocalizaciones de *Phaenostictus mcleannani*. Tiene dos objetivos específicos que corresponden a los dos capítulos de la tesis. El primer objetivo específico fue estudiar esta vocalización en un contexto donde hay conflicto entre emisor y receptor. Específicamente evalué si esta vocalización indica honestamente la calidad del emisor durante competencias vocales agresivas entre machos. El segundo objetivo específico fue estudiar la vocalización durante interacciones entre vecinos territoriales. En esta parte evalué si esta vocalización brinda información acerca de la identidad del emisor durante el reconocimiento de vecinos territoriales en los grupos de forrajeo.

Literatura citada

Amin, N., Grace, J. A. & Theunissen, F. E. 2004 Neural response to bird's own song and tutor song in the zebra finch field L and caudal mesopallium. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* **190**, 469-489.

Bard, S. C., Hau, M., Wikelski, M. & Wingfield, J. C. 2002 Vocal distinctiveness and response to conspecific playback in the Spotted Antbird, a Neotropical suboscine. *Condor* **104**, 387-394.

Beecher, M. D., Campbell, S. E. & Nordby, J. C. 2000 Territory tenure in song sparrows is related to song sharing with neighbours, but not to repertoire size. *Animal Behaviour* **59**, 29-37.

Catchpole, C. K. 1987 Bird Song, Sexual Selection and Female Choice. *Trends in Ecology & Evolution* **2**, 94-97.

Falls, J. B. 1982 Individual recognition by sounds in birds. In *Acoustic communication in birds*, vol. 2 (ed. M. E. H. Kroodsma D.E), pp. 237- 278. New York: Academic press.

Fedy, B. C. & Stutchbury, B. J. M. 2005 Territory defence in tropical birds: are females as aggressive as males? *Behavioral Ecology and Sociobiology* **58**, 414-422.

Forstmeier, W., Kempenaers, B., Meyer, A. & Leisler, B. 2002 A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**, 1479-1485.

Gil, D. & Gahr, M. 2002 The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology & Evolution* **17**, 133-141.

Goller, F. & Suthers, R. A. 1996 Role of syringeal muscles in controlling the phonology of bird song. *Journal of Neurophysiology* **76**, 287-300.

Hasselquist, D., Bensch, S. & vonSchantz, T. 1996 Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* **381**, 229-232.

Hopp, S. L., Jablonski, P. & Brown, J. L. 2001 Recognition of group membership by voice in Mexican jays, Aphelocoma ultramarina. *Animal Behaviour* **62**, 297-303.

Hurd, P. L. & Enquist, M. 2005 A strategic taxonomy of biological communication. *Animal Behaviour* **70**, 1155-1170.

Janicke, T., Hahn, S., Ritz, M. S. & Peter, H. U. 2008 Vocal performance reflects individual quality in a nonpasserine. *Animal Behaviour* **75**, 91-98.

Johnstone, R. A. 1996 Multiple displays in animal communication: 'Backup signals' and 'multiple messages'. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **351**, 329-338.

Johnstone, R. A. 1999 Signaling of need, sibling competition, and the cost of honesty. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 12644-12649.

Kroodsma, D. E. 1984 Songs of the Alder Flycatcher (Empidonax-Alnorum) and Willow Flycatcher (Empidonax-Traillii) Are Innate. *Auk* **101**, 13-24.

Kroodsma, D. E. & Konishi, M. 1991 A Suboscine Bird (Eastern Phoebe, Sayornis-Phoebe) Develops Normal Song without Auditory-Feedback. *Animal Behaviour* **42**, 477-487.

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.

Lovell, S. F. & Lein, M. R. 2004 Neighbor-stranger discrimination by song in a suboscine bird, the alder flycatcher, Empidonax alnorum. *Behavioral Ecology* **15**, 799-804.

Lovell, S. F. & Lein, M. R. 2005 Individual recognition of neighbors by song in a suboscine bird, the alder, flycatcher Empidonax alnorum. *Behavioral Ecology and Sociobiology* **57**, 623-630.

Mahler, B. & Tubaro, P. L. 2001 Relationship between song characters and morphology in New World pigeons. *Biological Journal of the Linnean Society* **74**, 533-539.

Mooney, R., Rosen, M. J. & Sturdy, C. B. 2002 A bird's eye view: top down intracellular analyses of auditory selectivity for learned vocalizations. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* **188**, 879-895.

Morton, E. S. & Derrickson, K. C. 1996 Song ranging by the dusky antbird, Cercomacra tyrannina: Ranging without song learning. *Behavioral Ecology and Sociobiology* **39**, 195-201.

Nowicki, S. & Searcy, W. A. 2004 Song function and the evolution of female preferences - Why birds sing, why brains matter. In *Behavioral Neurobiology of Birdsong*, vol. 1016, pp. 704-723.

Pfaff, J. A., Zanette, L., MacDougall-Shackleton, S. A. & MacDougall-Shackleton, E. A. 2007 Song repertoire size varies with HVC volume and is indicative of male quality in song sparrows (Melospiza melodia). *Proceedings of the Royal Society B-Biological Sciences* **274**, 2035-2040.

Price, J. J. 1999 Recognition of family-specific calls in stripe-backed wrens. *Animal Behaviour* **57**, 483-492.

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.

Scott-Phillips, T. C. 2008 Defining biological communication. *Journal of Evolutionary Biology* **21**, 387-395.

Searcy, W. A. 1992 Song Repertoire and Mate Choice in Birds. *American Zoologist* **32**, 71-80.

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

Seddon, N., Merrill, R. M. & Tobias, J. A. 2008 Sexually selected traits predict patterns of species richness in a diverse clade of suboscine birds. *American Naturalist* **171**, 620-631.

Seddon, N. & Tobias, J. A. 2006 Duets defend mates in a suboscine passerine, the warbling antbird (Hypocnemis cantator). *Behavioral Ecology* **17**, 73-83.

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.

High-pitched notes signal genetic diversity during vocal contests in ocellated antbirds

Animals use signals to assess the quality of rivals or mates. Increasing evidence suggest that biomechanically constrained acoustic features are costly to produce and therefore are honest indicators of singer quality. Furthermore, recent studies have found that some vocalization features reveal genetic quality, i.e. genetic diversity. To our knowledge, the possibility that biomechanically constrained acoustic features signal individual genetic diversity has not been explored. We evaluated this idea in a population of ocellated antbirds (*Phaenostictus mcleannani*) in Costa Rica. Recording and analyzing the aggressive vocalizations of radio tagged birds; we found individual variation and high repeatability in the highest frequency of their vocalization. An upper bound regression revealed that vocalization frequency (i.e. pitch) and duration were negatively correlated. This suggests that high-pitched vocalizations are costly to produce because they constrain the duration of the vocalization, probably because biomechanical limitations (e.g. muscle fatigue, oxygen limitation). We also found that individuals with higher genetic diversity produce higher pitched notes in their songs. And by means of playback experiments we showed that individuals increase the pitch of their vocalization during aggressive interactions. Overall, our results suggest that the ability to increase vocalization frequency is an honest indicator of male genetic quality and that it is used to settle male-male vocal contests. Given that sound production in different taxa shares some basic neuromuscular mechanisms, the assessment of singer quality through high-pitched notes can be a widespread phenomenon.

Key words: High frequencies, vocalization, genetic diversity, vocal contests

Individual variation in song production can affect individual fitness by means of mate choice and male-male competition (Searcy & Andersson 1986). Receivers assess the quality of conspecifics by the properties of their songs in order to maximize their own fitness, while senders produce songs designed to enhance their own reproductive success (Collins 2004; Searcy 2005). This can result in conflicts of interest between

senders and receivers, and theory predicts that songs must be under constraints to be honest indicators of quality (Gil & Gahr 2002; Grafen 1990; Zahavi 1975). Production of acoustic features that are difficult to produce can provide useful information to listeners about the quality of singers. Potential mates and rivals use individual variation in the performance of this acoustic features to judge male quality (Collins 2004).

Song performance can be constrained by the biomechanical limits of the vocal apparatus (Suthers 2004). The ability to produce one acoustic feature near its biomechanical limit can constrain the output of other important acoustic features of a vocalization (Cardoso et al. 2007; Lambrechts 1996; Podos 2004).This can produce a trade off between features and makes the performance of some acoustic features near its biomechanical limit costly because of decreases in the performance of other aspects of the vocalizations (Podos et al. 2004a). This kind of trade off has been documented in song birds, where the trilled part of the vocalization is an honest indicator of quality. There is a trade off between bandwidth and repetition rate due to biomechanical limitations. Experimental manipulations of songs have shown that 1) females prefer and 2) male competitors are more effectively repelled by songs closer to their biomechanical limit (Ballentine et al. 2004; Draganoiu et al. 2002; Illes et al. 2006).

How far individuals can push their biomechanical limits during song performance will depend on their phenotypic and ultimately genetic quality (Gil & Gahr 2002). A few correlations between song production and genetic diversity are reported in the literature (Marshall et al. 2003; Pfaff et al. 2007; Seddon et al. 2004). Genetic diversity can directly affect song production through developmental instability of the song production system (Pfaff et al. 2007) and indirectly by affecting the individual's overall condition and therefore its ability to sing (Moller 1991; Seddon et al. 2004). If genetic diversity affects the development of the vocal apparatus and/or bird's condition, song performance may be correlated with individual genetic diversity. Therefore difficult-to-produce acoustic features in the song could honestly reveal the quality of the singer.

Theoretical models about evolution of signalling systems where interest of the participants differ, predict that signals should have a cost to avoid invasion by cheaters

(Hurd & Enquist 2005). Empirical studies need to determine both the costs in the production of signals, and how these costs are related to the quality that they reveal. Increasing evidence suggests that biomechanically constrained acoustic features are honest indicators of singer quality(Podos 2004). Furthermore, recent studies have found that some vocalization features reveal genetic quality, i.e. genetic diversity(Marshall et al. 2003; Pfaff et al. 2007; Seddon et al. 2004). To our knowledge, the possibility that biomechanically constrained acoustic features signal individual genetic diversity has not been explored.

We used the aggressive vocalization of the ocellated antbird (*Phaenostictus mcleannani*) during male-male vocal contests to evaluate 1) if individual variation in performance of biomechanically constrained acoustic features reveal male genetic diversity and 2) if these traits are used to settle ritualized male-male aggressive interactions. We first determined the degree of variation among individuals and repeatability in different acoustic features in particular individuals. We identified acoustic features that are costly to produce by analyzing possible trade offs between temporal and the spectral features of the vocalization as in (Cardoso et al. 2007; Janicke et al. 2008; Podos 1997). We then evaluated the existence of correlations between individuals' genetic diversity and presumably constrained aspects acoustic features of their vocalizations. Finally, we used playback experiments to show how individuls use variation in acoustic features to settle ritualized aggressive interactions.

METHODS

Study population

We studied the ocellated antbird, *Phaenostictus mcleannani* (Thamnophilidae). These are Neotropical obligate antbirds, that pair for several years and congregate at swarms of army ants to feed on arthropods that attempt to escape from these ants (Willis & Oniki 1978). We focused on a vocalization called "loud song", which is produced year round by both sexes. It is usually used to attract a mate to a recently discovered swarm of ants and as departure call when one member of the mated pair is about to leave the swarm of ants, and in agonistic interactions between males (Willis 1973). The loud

song is composed of stereotyped elements that rise in pitch, relative amplitude and repetition rate during the beginning and middle part of the song and then decrease at the end of the vocalization (Fig1).

We conducted three 14-week field seasons at La Selva Biological Station Costa Rica (10°25' N, 84°01' W) from February 2005 to September 2006. We captured ocellated antbirds opportunistically using mist-nets set up at army ant colonies within a study area of 392 ha. From 3 to 10 mist-nets were opened every 2-3 days between 5:00 am and 9:00 am during 3 weeks. A small sample of blood was collected from each individual for molecular sexing and genetic analysis. All birds were uniquely banded, and 10-19 individuals were outfitted with radio transmitters in each field season to follow them to army ants swarms (for details on genetic analysis, tagging, and tracking see Chaves-Campos & DeWoody submitted).

Vocalization recording and acoustic analysis

We recorded vocalizations of males during spontaneous aggressive interactions and during simulated aggressive dominance challenges (see below), using a Senheiser Mkh 65 microphone that was attached to a Sony minidisk recorder. Songs were digitized with no compression with a 44.1 Khz. sampling rate. Spectrograms were generated using Avisoft SASLabPro Version 4.0c. The loud songs were automatically filtered at half the Nyquist frequency. Spectrograms were produced using the broad-band (323 Hz) filter settings in Avisoft using a flat top window, frame size 54%, overlap 87.5% and Fast Fourier transformation 1024. These spectrogram parameters resulted in a spectral resolution of 43 Hz and a temporal resolution of 3 ms. From each spectrogram, we measured the spectral and temporal variables indicated in Figure 1. We chose these measurements because in we believe they adequately describe the structural modulations and variations in this vocalization in this species.

Individual variation

We used analysis of variance (ANOVA) to determine variation among 13 individuals in each variable. We used individual as a factor and the acoustic feature as

the dependent variable. Each acoustic variable was tested for normality and homoscedasticity. The repeatabilities for each acoustic feature were estimated as the intra class correlation coefficients in the ANOVA (Lessells & Boag 1987).

Biomechanical constraints in the vocalization

We analyzed correlations between spectral and temporal parameters of the vocalization to identify possible biomechanical constraints. To evaluate the existence of a tradeoff between frequency and duration we conducted an upper bound regression (Podos 1997; Price et al. 2006). We arbitrary binned songs in 500 Hz categories and from each category we chose the vocalization with the highest duration. The upper bound regression was performed only with these values in order to exclude the vocalizations in which individuals did not produced frequencies as high or as long as they can physically (Podos 1997, Price *et al.* 2006).

Correlation with genetic diversity

We calculated individual average values for each of the vocalization variables and used them in a regression analysis that included estimates of individual genetic diversity for 10 individuals. We calculated three widely used indices of individual genetic diversity (reviewed by (Aparicio et al. 2006)): internal relatedness (IR) homozygosis by loci (Hl); standardized heterozygosis (HS). These indices were calculated using 9 polymorphic microsatellite loci (average: 7.4 alleles per locus), developed for ocellated antbirds (Chaves-Campos and DeWoody, *submitted*).

Variation in song features during playback experiments

We recorded loud songs produced by individuals spontaneously interacting with their mates at ant swarms. These were compared with the vocalizations recorded after exposing the same individuals on different days to simulated dominance challenges using playbacks. Ocellated antbirds show site-related dominance, in which the bird that holds the dominance in which the army ant swarm is located on particular day is the only individual that answers playbacks (Chaves-Campos and Araya-Ajoy, unpublished

data). Playback experiments were conducted while individuals were foraging at a swarm, to simulate an aggressive challenge to the dominant male. The stimulus was broadcasted from a Poly planar MA 840 speaker (Frequency Response: 70Hz to 14KHz) located 10 to 20 meters from the focal individual. The aggressive stimulus consisted of the vocalization of an individual broadcasted in three periods of 1 minute which contained 5 vocalizations. These one minute periods of vocalizations were alternated by one minute intervals of silence. We used the vocalizations of five different individuals to conduct the playbacks to avoid pseudoreplication (Kroodsma 1990). We used generalized linear mixed models in SAS 9.1 (Proc Mixed or Proc Glimmix for normal or non-normal error distributions respectively) to test for differences between vocalizations produced in response to aggressive simulations and those produced spontaneously. We used the context in which the vocalization was produced as a fixed effect and the individual that produced the vocalization as a random effect (repeated measure).

RESULTS

Individual variation

Vocalizations with the best signal-to-noise ratio from 10 different individuals were analyzed (mean= 6 per individual, range 4-10). Individuals differed in all acoustic features except bandwith (Table 1).The repeatability of the acoustic features ranged from 0.30-0.75. The features with the highest repeatabilities were the frequency of the middle element, the frequency in the highest element, and the duration of the vocalization (Table 1).

Song performance

The upper bound regression revealed a negative correlation between the duration and the frequency of the vocalizations ($y= -1.52x+14.20$, $r^2=0.83$, $p=0.01$; Fig 2). This suggests that high frequencies cannot be sustained for long periods of time resulting in a tradeoff between temporal and spectral features of the vocalization.

Correlation with genetic diversity

We found strong correlations between each of the three measures of an individual's genetic diversity and two spectral variables of the aggressive vocalizations (Fig 3). Specifically, there was a strong negative correlation with the fundamental frequency in the middle note of the vocalization with (IR: $r^2 = 0.62$, p= 0.004; Hl: $r^2 = 0.68$, p=0.001; Hs: $r^2 = 0.60$, p=0.005). There were similar correlation with the highest fundamental frequency in the vocalization (IR: $r^2 = -0.45$, p=0.03; Hl: $r^2 = -0.45$, p=0.02; Hs $r^2 = 0.44$, p=0.03). This pattern allows interpreting that increased genetic diversity is linked to the production of higher frequencies.

Variation in vocalization features during playback experiments

We compared 109 vocalizations recorded from 15 individuals in natural aggressive interactions or as responses to simulated challenges of dominance. We interpreted the response of the individulas as aggressive because they flew to the speakers, and when perched started to perform aggressive displays as described by Willis (1973). Individuals responding to playbacks produced higher pitched and longer vocalizations compared to those produced by the same individuals in the absence of a vocal challenge (Table 2). Individuals raised the pitch of all acoustic features when responding to the challenge, except the frequency of the lowest element and the frequency in the final element of the vocalization. Individuals also produced longer vocalizations and reached the maximun frequency faster during the response to playbacks. This indicates that the pitch and the duration of the vocalization are used to settle male-male vocal contests.

DISCUSSION

Ocellated antbirds varied individually in the highest frequency they produced in aggressive vocalizations. High-pitched vocalizations cannot be sustained for a long time and individuals with higher genetic diversity produced higher pitched notes. Also the pitch of the vocalization increases during aggressive interactions. Overall this suggests

that the ability to increase the frequency in the aggressive vocalizations is an honest indicator of male quality and is used to settle ritaulized aggressive interactions.

As other suboscines, antbirds are thouhgt to lack a learned song components (Kroodsma 1984). Thus the differences among individuals and the high repeatabilities of the vocalization characteristics has to be caused by individual differences in the nervous system, vocal anatomy and physical condition. Furthermore, our results suggests that among-individual variation in vocalizations is caused by individual differences in the ability to push the biomechanical limits of their vocal apparatus.

Song performance

When we performed the upper bound regression we found a negative correlation between the duration and the frequency of the vocalization (Fig 2). We interpret this result as a tradeoff caused by biomechanical constraints in song production (Podos 2004). The difficulty of producing high-pitched notes could be partially explained by the neuromuscular control of bird song. The main source of sound production in birds is the syrinx. Within this organ, the tension of the labia mainly determines the fundamental frequency of the notes (Suthers et al. 1999). The activity of the muscles controlling the labia increases exponentially with increasing fundamental frequencies(Goller & Suthers 1996). It has been suggested that producing high frequencies, as the frequencies produced by ocellated antbirds, requieres substantial force and contraction effort from these muscles(Collins 2004). Higher frequency sounds are also costly to produce as the sound energy power varies with the square of frequency (Galeotti et al. 1997).

Individual differences in respiration ability could explain the tradeoff between temporal and spectral features. The part of the song with the highest pitch notes are highest also has the highest note repetition rate (Fig 1). The respiratory rhythm of a singing individual determines the basic temporal pattern of a song (Goller & Suthers 1996). Song is produced during expirations, so there are brief inspiratory breaths between syllables to prevent the bird from running out of air. There is a net loss of respiratory volume during the production of parts of the song with high repetition rate called trills in some species (Suthers et al. 1999). The temporal pattern of the part we are

focusing on closely resemble trills and probably demands similar respiratory demands. The combining effect of high activity in the ventral syrinx muscles, and running out of air during the rapid production of notes could lead to muscular fatigue (Lambrechts 1996; Suthers et al. 1999). This interpretation is congruent with our result that high-pitched vocalizations cannot be sustained for long periods of time.

Our interpretation is also complemented by previous work that shows that the fundamental frequency of a song is also influenced by how widely the beak is opened. Birds open the beak wider during high frequencies than during low frequencies (Podos et al. 2004b). The fast element repetition rate of this part of the song in *P. mcleannani* (Fig 1) suggests that these birds should open and close their beaks rapidly during its production. Overall the combination of these basic aspects of the neuromuscular control on song production (muscular effort, respiratory rate, and beak-opening rate) suggests to us that the high pitched part of the vocalization is biomechanically constrained.

Correlation with genetic diversity

Reduced genetic diversity can affect individual fitness negatively due to increased homozygosis either by allowing the expression of recessive deleterious alleles or by reducing heterozygote advantage (Keller & Waller 2002). The same mechanisim could explain correlations between song traits and genetic diversity (Marshall et al. 2003; Pfaff et al. 2007; Seddon et al. 2004).

If genetically more diverse individuals experience less developmental stress in the vocal apparatus, a correlation between genetic diversity and high frequency could arise in ocellated antbirds. Alternatively given that heterozygosity can affect the overall condition of individuals (Acevedo-Whitehouse et al. 2003; Amos et al. 2001; Reid et al. 2003), birds in better condition could produce higher pitched songs. The two mechanisms are not mutually exclusive and male genetic quality might be reflected by his song due to multiple proximate causes of the correlation.

Since microsatellite makers should not reflect genome-wide heterozygosity, the origin of the correlation between song frequency and heterozygosity should be explained (DeWoody & DeWoody 2005). Small number of microsatellite loci are expected to

reflect overall individual heterozygosity when there are high levels of linkage disequilibrium, usually as a product of inbreeding (Lynch & Walsh 1998), or when microsatellites are tightly linked to coding genes (Hansson et al. 2001). Given that the population we studied is not inbreed (F_{is} = 0.01; P=0.73; permutation test; n=57 individuals) our interpretation of the results is that at least some of the microsatellites cosegregate with genes or gene complexes that either directly affect the capacity of producing song in ocellated antbirds or that influence individual health and vigor, such as Mhc genes (Zelando and Edwards 2002).

Variation in vocalization features during playback experiments

During aggressive interactions ocellated antbirds produce vocalizations of higher pitch and longer duration than when singing spontaneously. This suggests that males use these traits to assess the quality of contenders (Collins 2004). This result supports our hypothesis that high-pitched notes are difficult to produce. Producing signals near their maximum biomechanical limit is specially challenging for singers and therefore individuals should perform these versions of the signal only when necessary (Price et al. 2006).In addition, the same features we interpret having tradeoff were involved in the difference in vocalizations produced during responses to simulated challenges and vocalizations produced in other contexts; this fits with our assumption that the constraint we found was between two variables important in song performance. Although we have not demonstrated that high-pitched and longer vocalizations are more effective at repelling males, our experimental design provide evidence suggesting their importance during male-male vocal contests(Collins 2004).

Evolutionary relevance

We conclude that the ability to produce high-pitched vocalizations is under intrasexual selection in this specie. This could have important evolutionary implications explaining the maintenance of genetic variance in sexually selected traits and how sexual selection shapes signal design. In the context of the ongoing debate on why directional selection does not deplete genetic variation in sexually selected traits, the

correlation between genetic diversity and song performance supports the idea that sexually selected traits can be linked to heterozygosity rather than to additive genetic variance (Brown 1997). This mechanism would maintain the variance of the trait even under the influence of directional selection, maintaining its usefulness to assess male quality.

According to the environmental adaptation hypothesis; efficiency in transmission of the signal should favor low frequencies and low rates of element repetition in densely vegetated habitats (Slabbekoorn 2004). The high frequency and high element repetition rate of the part of the song we are focusing has characteristics with serious attenuation and degradation problems in densely vegetated habitats like tropical rain forests (Slabbekoorn 2004). A reasonable explanation for the maintenance of these characteristics in the vocalization of this species is that intra-sexual selection counterbalances negative selection caused by environmental constraints in signal transmission. Moreover, only males in good condition can deal with the attenuation problems of high pitch sounds. The quality of the male is revealed because of the relation between the mechanism underlying song production and male quality, but also because the extra cost in the efficiency of transmision of high frequencies.

There is evidence that high frequencies can reveal singers' quality across taxa. It has been demonstrated that songs of dominant roosters have higher fundamental frequencies than subordinates and that this is an important cue during ritualized aggressive interactions among males (Leonard & Horn 1995). It is also known that human voice loses tonal quality and phonetic information at high frequencies, a problem that quality soprano singers overcome by training their gifted vocal apparatus (Joliveau et al. 2004). Given that sound production in different taxa share some basic neuromuscular mechanism, the use of high pitched notes to assess the quality of possible mates or rivals could be a widespread phenomenon.

REFERENCES

Acevedo-Whitehouse, K., Gulland, F., Greig, D. & Amos, W. 2003 Disease susceptibility in California sea lions. *Nature* **422**, 35-35.

Amos, W., Wilmer, J. W., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001 The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 2021-2027.

Aparicio, J. M., Ortego, J. & Cordero, P. J. 2006 What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology* **15**, 4659-4665.

Ballentine, B., Hyman, J. & Nowicki, S. 2004 Vocal performance influences female response to male bird song: an experimental test. *Behavioral Ecology* **15**, 163-168.

Brown, J. L. 1997 A theory of mate choice based on heterozygosity. *Behavioral Ecology* **8**, 60-65.

Cardoso, G. C., Atwell, J. W., Ketterson, E. D. & Price, T. D. 2007 Inferring performance in the songs of dark-eyed juncos (Junco hyemalis). *Behavioral Ecology* **18**, 1051-1057.

Collins, S. 2004 Vocal Fighting and Flirting.The function of bird song. In *Nature's Music. The Science of Bird Song* (ed. P. Marler, H. Slabbekorn), pp. 39-78
Elsevier Academic Press.

DeWoody, Y. D. & DeWoody, J. A. 2005 On the estimation of genome-wide heterozygosity using molecular markers. *Journal of Heredity* **96**, 85-88.

Draganoiu, T. I., Nagle, L. & Kreutzer, M. 2002 Directional female preference for an exaggerated male trait in canary (Serinus canaria) song. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**, 2525-2531.

Galeotti, P., Saino, N., Sacchi, R. & Moller, A. P. 1997 Song correlates with social context, testosterone and body condition in male barn swallows. *Animal Behaviour* **53**, 687-700.

Gil, D. & Gahr, M. 2002 The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology & Evolution* **17**, 133-141.

Goller, F. & Suthers, R. A. 1996 Role of syringeal muscles in controlling the phonology of bird song. *Journal of Neurophysiology* **76**, 287-300.

Grafen, A. 1990 Biological Signals as Handicaps. *Journal of Theoretical Biology* **144**, 517-546.

Hansson, B., Bensch, S., Hasselquist, D. & Akesson, M. 2001 Microsatellite diversity predicts recruitment of sibling great reed warblers. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 1287-1291.

Hurd, P. L. & Enquist, M. 2005 A strategic taxonomy of biological communication. *Animal Behaviour* **70**, 1155-1170.

Illes, A. E., Hall, M. L. & Vehrencamp, S. L. 2006 Vocal performance influences male receiver response in the banded wren. *Proceedings of the Royal Society B-Biological Sciences* **273**, 1907-1912.

Janicke, T., Hahn, S., Ritz, M. S. & Peter, H. U. 2008 Vocal performance reflects individual quality in a nonpasserine. *Animal Behaviour* **75**, 91-98.

Joliveau, E., Smith, J. & Wolfe, J. 2004 Acoustics - Tuning of vocal tract resonance by sopranos. *Nature* **427**, 116-116.

Keller, L. F. & Waller, D. M. 2002 Inbreeding effects in wild populations. *Trends in Ecology & Evolution* **17**, 230-241.

Kroodsma, D. E. 1984 Songs of the Alder Flycatcher (Empidonax-Alnorum) and Willow Flycatcher (Empidonax-Traillii) Are Innate. *Auk* **101**, 13-24.

Kroodsma, D. E. 1990 Using Appropriate Experimental-Designs for Intended Hypotheses in Song Playbacks, with Examples for Testing Effects of Song Repertoire Sizes. *Animal Behaviour* **40**, 1138-1150.

Lambrechts, M. 1996 Organization of Bird Song and Constraints in Performance. In *Ecology and Evolution of Acoustic Comunication in Birds* (ed. D. Kroodsma, E. Miller), pp. 314-318: Comstock Publishing Associates.

Leonard, M. L. & Horn, A. G. 1995 Crowing in Relation to Status in Roosters. *Animal Behaviour* **49**, 1283-1290.

Lessells, C. M. & Boag, P. T. 1987 Unrepeatable Repeatabilities - a Common Mistake. *Auk* **104**, 116-121.

Marshall, R. C., Buchanan, K. L. & Catchpole, C. K. 2003 Sexual selection and individual genetic diversity in a songbird. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S248-S250.

Moller, A. P. 1991 Parasite Load Reduces Song Output in a Passerine Bird. *Animal Behaviour* **41**, 723-730.

Pfaff, J. A., Zanette, L., MacDougall-Shackleton, S. A. & MacDougall-Shackleton, E. A. 2007 Song repertoire size varies with HVC volume and is indicative of male quality in song sparrows (Melospiza melodia). *Proceedings of the Royal Society B-Biological Sciences* **274**, 2035-2040.

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

Podos, J., Huber, S. K. & Taft, B. 2004a Bird song: The interface of evolution and mechanism. *Annual Review of Ecology Evolution and Systematics* **35**, 55-87.

Podos, J., S. Nowicki. 2004 Performance Limits in Bird Song. In *Nature's Music. The Science of Bird Song* (ed. P. Marler, H. Slabbekorn), pp. 318-343. Cambridge: Elsevier Academic Press.

Podos, J., Southall, J. A. & Rossi-Santos, M. R. 2004b Vocal mechanics in Darwin's finches: correlation of beak gape and song frequency. *Journal of Experimental Biology* **207**, 607-619.

Price, J. J., Earnshaw, S. M. & Webster, M. S. 2006 Montezuma oropendolas modify a component of song constrained by body size during vocal contests. *Animal Behaviour* **71**, 799-807.

Reid, J. M., Arcese, P. & Keller, L. F. 2003 Inbreeding depresses immune response in song sparrows (Melospiza melodia): direct and inter-generational effects. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 2151-2157.

Searcy, W. A. & Andersson, M. 1986 Sexual Selection and the Evolution of Song. *Annual Review of Ecology and Systematics* **17**, 507-533.

Searcy, W. A. a. N., S. 2005 *The Evolution of Animal Communication: Reliability and Deception in Signaling*. New Jersey: Princeton University Press.

Seddon, N., Amos, W., Mulder, R. A. & Tobias, J. A. 2004 Male heterozygosity predicts territory size, song structure and reproductive success in a cooperatively breeding bird. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 1823-1829.

Slabbekoorn, H. 2004 Singing in the Wild. The Ecology of Bird Song. In *Nature's Music. The Sicence of Bird Song* (ed. P. Marler, H. Slabbekorn), pp. 178-205: Elsevier Academic Press.

Suthers, R. 2004 How birds sing and why it matters. In *Nature's Music. The Science of Bird Song* (ed. P. Marler, H. Slabbekorn), pp. 272-295 Elsevier Academic Press

Suthers, R. A., Goller, F. & Pytte, C. 1999 The neuromuscular control of birdsong. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **354**, 927-939.

Willis, E. O. & Oniki, Y. 1978 Birds and Army Ants. *Annual Review of Ecology and Systematics* **9**, 243-263.

Zahavi, A. 1975 Mate Selection - Selection for a Handicap. *Journal of Theoretical Biology* **53**, 205-214.

Table 1. ANOVA results for the variation among individuals vocalization.
r = repeatability measured as the intraclass correlation. See Fig 1 for definition of variables.

Acoustic feature	F	df	p	r
F. first element	4.35	12/78	<0.001	0.57
F. last element	3.93	12/78	<0.001	0.62
F. middle element	7.30	12/78	<0.001	0.75
F. highest element	6.03	12/78	<0.001	0.70
F. lowest element	4.28	12/78	<0.001	0.60
Max. F	5.53	12/78	<0.001	0.68
Bandwidth	1.92	12/78	0.066	0.30
Duration	5.15	12/78	<0.001	0.66
Time to highest F	3.86	12/78	0.001	0.58
Time to max F	2.60	12/78	0.014	0.43
Elements after highest F	4.83	12/78	<0.001	0.64

Table 2. Variation in the in **acoustic** features when individuals sing spontaneously and when elicited by a playback **stimulus**. +/- standard deviation, all tests have 1/105 degrees of freedom.

| Acoustic feature	Spontaneously		Experiments		Tests	
	Mean	+/-	Mean	+/-	F	p
F. first element	2.95	0.07	3.09	0.07	5.87	0.01
F. last element	3.16	0.07	3.07	0.06	1.28	0.26
F. middle element	3.72	0.1	4.03	0.1	21.21	<0.01
F. highest element	4.55	0.1	4.8	0.9	19.88	<0.01
F. lowest element	2.68	0.07	2.66	0.06	0.18	0.67
Max. F	3.69	0.1	3.90	0.09	10.27	<0.01
Bandwidth	1.86	0.08	2.16	0.07	20.30	<0.01
Duration	3.81	0.25	4.74	0.22	19.18	<0.01
Time to highest F	2.87	0.14	3.13	0.13	5.34	0.02
Time to max F	2.37	0.19	1.82	0.16	10.67	<0.01
Elements after highest F	1.24	0.36	3.04	0.30	30.13	<0.01
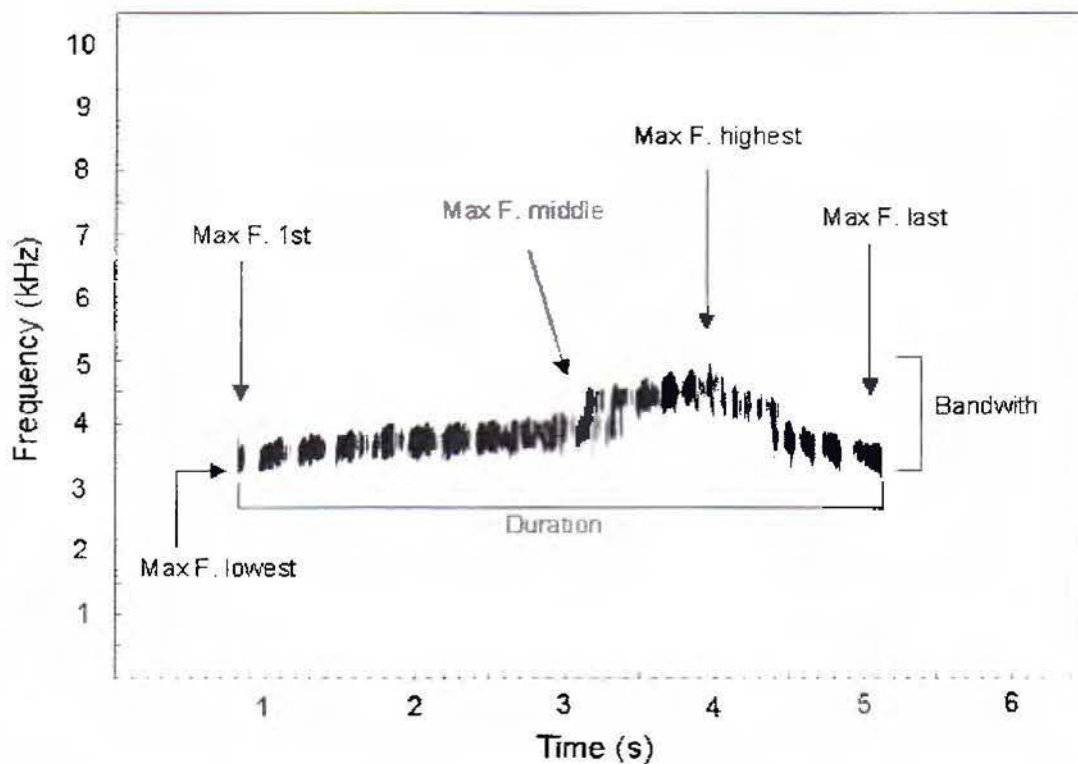
Figure 1. Annotated spectrogram of the "loud song" of an ocellated antbird male. Loudsong structure was quantified using on-screen cursors to measure the following time and frequency traits: (1) Maximum frequency of the first element (Max F. 1st) (2) Maximum frequency of middle elements (Max F. mid), (3) Maximum frequency of the last element (F. last), (4) Maximum frequency of the element with highest frequency (Max F. highest), (5) Maximum frequency of the element with the lowest frequency (Max F. Lowest), 6) maximum frequency of the entire vocalization (Max F.)*, (7) vocalization bandwidth (bandwith), (8) vocalization duration (Duration), (9) time elapsed until the element with the highest frequency was produced*, (10) time elapsed until the element with the maximum frequency of the entire vocalization was produced*, (11) number of elements produced after the element with the highest frequency*. Maximum frequency refers to the frequency with most energy in the element.

* Measurements not shown in the annotated spectrogram.
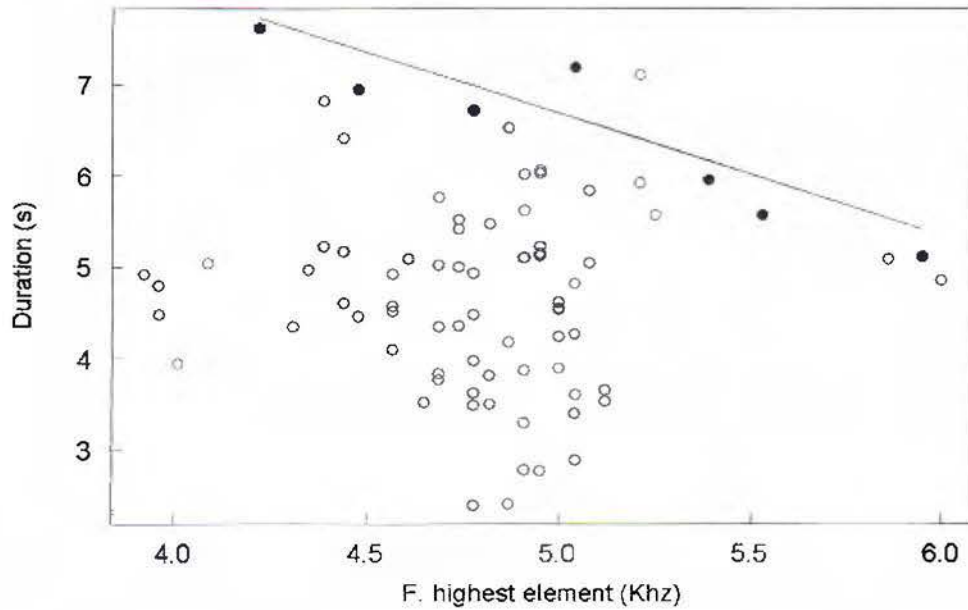
Figure 2. Correlation between the frequency of the element with highest pitch and vocalization duration. Filled circles show the values used in the upper bound regression.
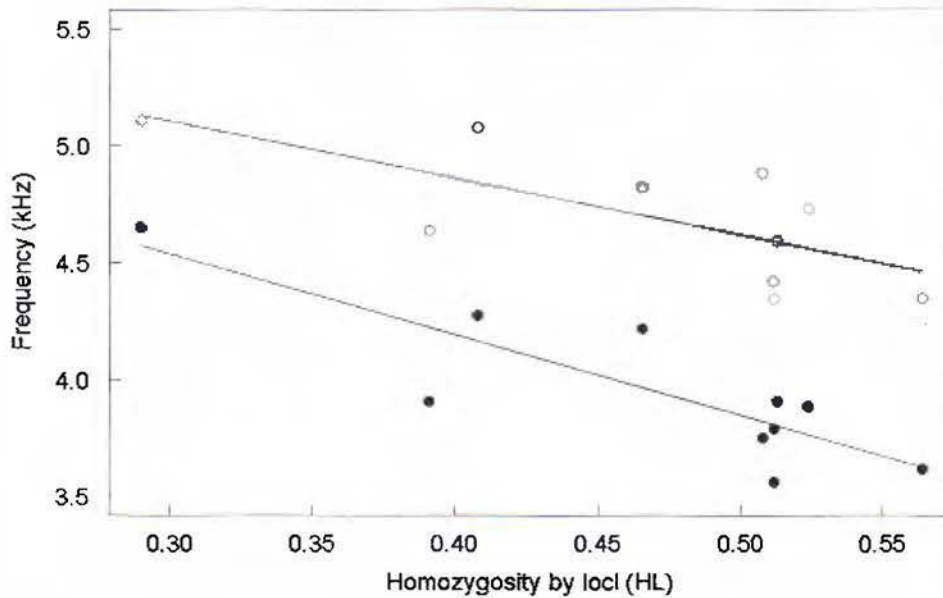

Figure 3. Correlation between the fundamental frequency of the middle element (open circles) and the fundamental frequency of the highest element (filled circles) with genetic diversity (measured as homozigosity by loci).

Vocal distinctiveness allows individual recognition in reciprocal tolerance relations of an obligate army ant-following bird

Discriminating between conspecifics is very important in the formation of a wide array of social stable groups of animals. Complex social interactions can favor individual recognition, especially when there are repeated interactions between individuals with deferring roles/intentions. We studied individual recognition in the Ocellated antbird, a neotropical bird that has reciprocal tolerance relations with their neighbors that allow them to depend exclusively from the arthropods that are flushed by army ants. Its known that benefits from delayed reciprocity relations can only be achieved if individual recognition is possible, thus we evaluated if vocal distinctiveness of the lodsoung in this species allows acoustic discrimination of conspecifics in stable foraging assemblages. We found that individuals are capable to discriminate the vocalizations of neighbors (i.e. a bird. that is nearest neighbor to the focal bird and usually feed together at swarms) and strangers (i.e. a bird that roosts more than three roosting areas away to the focal bird and therefore rarely or never feed together at swarms). The results suggest that benefits attained by discriminating between conspecifics could have selected for the phenotypic variability observed in the contact loudsongs of this specie and the ability to acoustically discriminate between individuals.

Key words: identity, signals, individual recognition, phenotypic variability, vocalizations.

Discriminating between conspecifics is necessary in the formation of many stable social groups in animals (Tibbetts & Dale 2007). If being recognized is beneficial, selection should favor individuals who actively broadcast information about their identity to facilitate accurate recognition. This will be the case when there is the possibility of costly confusion between the signaler and another individual (Dale et al. 2001). Even if the signaler is not selected to broadcast information about its identity, the ability from listeners to use existent phenotypic variation to recognize individuals could

evolve. Complex social interactions can favor individual recognition (IR), specially when there are repeated interactions between individuals with deferring roles/intentions (Tibbetts 2004).

One of the most documented cases of IR is the discrimination of territorial neighbors. IR is thought to reduce the energetic costs of territorial defense by enabling territory holders to focus their aggressive efforts only to individuals that represent a treat for them (Temeles 1994). Evidence has been found in aquatic invertebrates (Detto et al. 2006), mammals (Kokko et al. 2001) and lizards (Husak & Fox 2003). Moreover, acoustic neighbor recognition has been demonstrated in frogs (Bee & Gerhardt 2002), fish (Myrberg & Riggio 1985) and birds (Falls 1982). Benefits attained by receivers as a product of discriminating between conspecifics have been identified in other contexts. IR has been proposed as a mechanism to explain the dominance relationships generated through agnostic interactions and maintained based on the memory of the outcomes of past encounters (Dugatkin & Earley 2004; Karavanich & Atema 1998). Moreover, benefits from delayed reciprocity relations can only be achieved if IR is possible (Crowley et al. 1996; Trivers 1971).

In contrast of the benefits that individuals gain discriminating between conspecifics, the benefits that signaler can obtain by signaling their own identity have received little attention. Selection on signalers to be memorably different might provide an underappreciated selective mechanism that increases phenotypic variability. Theoretical models shows how identity signals can evolve via negative frequency dependent selection if individuals with rare phenotypes are more distinctive and less likely to be confused with others (Dale et al. 2001). Indeed selection to signal identity might explain the highly variable olfactory signals in many social mammals (Brennan & Kendrick 2006), variation in color of some birds (Dale et al. 2001), the complex acoustic signals in many primates (Rendall et al. 1996) and the distinctive contact call among social birds (Falls 1982).

Identity signals can evolve in social behaviours as territoriality (Temeles 1994), reciprocal altruism (Crowley et al. 1996), monogamous pairing (Vignal et al. 2008) and dominance (Dugatkin & Earley 2004). Although identity signals are probably

widespread in nature, much more data on the benefits of distinctiveness are needed to fully understand the circumstances that favor identity signaling (Tibbetts & Dale 2007).To investigate individual recognition in a novel context we studied the loudsong of *Phaenostictus mcleannani*. Ocellated antbirds are obligate army ant-followers that do not defend exclusive territories. They vocalize loudly to their mates when they just arrive to a swarm or when they are about to leave a swarm (Willis 1973). Occasionally males vocalize back and forth to other individuals at army ant swarms for a few minutes until the conflict escalates to physical aggression and one male chases the other one away (pers. obs.). We have previously shown that these birds hold areas of local dominance and that nearest neighbors reciprocally grant access to the best part of swarms to each other when army ant swarms occur in their dominance area (Chaves-Campos et al. in preparation). Reciprocal tolerance between neighboring pairs creates relatively stable feeding groups through time, where not all individuals are equally tolerated. It is known IR is key in the evolution of reciprocal relations in contexts with dynamic interactions of many individuals (Crowley et al. 1996) and given that this species lives in densely vegetated environments with dim light conditions the evolution of acoustic identity signals are expected.

We evaluated if contact loudsongs of ocellated antbirds (*P. mcleannani*) signal individual identity. Acoustic discrimination could not evolve if vocalizations do not vary consistently among individuals (Falls 1982), thus we expected individual distinctiveness in the contact loudsongs. Reliable identity cues should have consistent variation among individuals and should be relatively inflexible between contexts. Given that vocalizations are multicomponent signals we studied, the variation among individuals and the stability through contexts (aggressive and contacting their mate) from different acoustic features. We then proceeded to test whether individuals can discriminate between vocalizations of a neighbor (i.e. a bird. that is nearest neighbor to the focal bird and therefore usually feed together at swarms) or a stranger (i.e. a bird that roosts more than three roosting areas away to the focal bird and therefore rarely or never feed together at swarms).

METHODS

Vocalization recording and acoustic analysis

We recorded loudsongs (sensu Willis 1973) of individuals in two different contexts: 1) Contacting its mate when arriving or leaving a swarm front and 2) individuals responding aggressively to play backs simulating a dominance challenge. Loud songs in both contexts were recorded using a Senheiser Mkh 65 microphone, attached to a Sony minidisk recorder. Songs were digitized with no compression with a 44.1 sampling rate. Spectrograms were generated using Avisoft SASLabPro Version 4.0c. The loud songs were automatically filtered at half the Nyquist frequency. Spectrograms were produced using the broad-band (323 Hz) filter settings in Avisoft using a flat top window, frame size 54%, overlap 87.5% and Fast Fourier transformation 1024. These spectrogram parameters resulted in a spectral resolution of 43 Hz and a temporal resolution of 3 ms. From the spectrograms we measured the spectral and temporal traits showed in figure 1. We chose these measurements because in our opinion they describe well the structural modulation and variation of the vocalization.

Vocal distinctiveness

We performed a discriminant function analysis to evaluate vocal distinctiveness of vocalizations produced when contacting with their mate arriving or leaving ant-swarms. To compare distinctiveness between contexts, we performed another discriminant function analysis to evaluate distinctiveness in the vocalizations in aggressive contexts. We used as a criterion of vocal distinctiveness the percentage of correct classifications with the entire data set and using a cross validation procedure.

Individual variation and context stability

We used generalized linear mixed models in SAS 9.1 (Proc Mixed) to evaluate the effect of the identity of the singer that produced the vocalization and the effect of the context were it was produced for each vocalization's acoustic features. We used

vocalizations of 15 individuals, departures from normality were assessed using quantile-quantitle plots and normality tests. Overdisperssion was assessed by inspection of the variance inflation factor (≤ 1 in all cases). The model has as fixed effect the context were the vocalization was produced (contacting mate or aggressive interaction), the identity of the singer as a random factor and the dependent variable was each of the acoustic features measured. We derived an ANOVA table from the model to analyze the contribution of each factor of the model to the variation in the data.

Conspecifics acoustic discrimination

We simulated accosting challenges of dominance to focal birds at swarms using the vocalization of either a neighbor or a stranger. We recorded vocalizations of individuals of known identity producing loudsongs to contact with their mate for the neighbor stimulus. And we used loud songs recorded by other researchers in the vicinities of La Selva for the stranger stimulus. Vocalizations were digitalized and standardized to the same amplitude, song rate, and total time, and broadcasted around 20 meters from the focal bird from a Poly planar MA 840 speaker (Frequency Response: 70Hz to 14KHz) connected to a CD player speaker. One researcher, concealed in a hunting blind, played back the stimulus and recorded the acoustic response of the focal bird during a 10-min period after the stimulus ended (none response to playbacks lasted longer), using a Senheiser Mkh 65 microphone attached to a Sony minidisk recorder. The stimulus consisted of 5 minutes: 1 minute which contained 5 vocalizations alternated by one minute of silence; the amplitude corresponded to the average value measured from field events. A second researcher indentified the bird emitting the response from a hunting blind located 10 m away from the speaker. A typical response consisted of only one male bird leaving the swarm to loud sing at the speaker for several minutes. The distance from the swarm to the center of the roosting area of each focal bird was measured to control for its potential confounding effect with Arcview animal movement (Hooge & Eichenlaub 1997) using geo-referenced landmarks of the biological station.

We collected repeated measures of responses from different birds to a neighbor or a stranger stimulus, sometimes on consecutive days. We measured 1) the number of songs that the individual produced answering to the stimulus. 2) The time elapsed from the beginning of the experiment and the response of the focal individual. 3) The total duration of the focal individual response and 4) the number of harsh elements produced in the vocalizations. We used generalized linear mixed models in SAS 9.1 (Proc Mixed or Proc Glimmix for normal or non-normal error distributions respectively) to evaluate if individuals response were different between vocalizations of neighbors and strangers. Departures from normality were assessed using quantile-quantitle plots and normality tests. Overdisperssion was assessed by inspection of the variance inflation factor (≤ 1 in all cases). The model had treatment (neigbour or stranger) as fixed effect and as random factors the identity of the singer and the distance from the roosting area, the dependent variable was the first principal component of variance of the response variables.

RESULTS

Vocal distinctiveness

A discriminant function analysis revealed that vocalizations produced contacting their mate can be correctly classified 90.09 % using all the data and 63.6% of the times using a cross validation procedure($F_{120/550} = 4.35$ p= <0.001). Meanwhile vocalizations during aggressive challenges were not as distinctive (DFA correct classifications 73%, cross validated 52%; $F_{120/185} = 6.46$, p=<0.001). Only four of the ten acoustic features measured were necessary to get the best classification functions for the contact vocalizations meanwhile aggressive vocalizations needed the ten acoustic features to get the best classification functions (Table 1).

Individual variation and context independence

Our model demonstrated that both individual identity and context had an effect in the variation of the vocalizations (Table 1). The identity of the singer had a significant effect on the variation of the vocalizations for all acoustic features measured. The only

acoustic features that were not affected by the context of production were the fundamental frequency of the final and lowest element in the vocalization (Table 1).

Vocal discrimination between familiar and unfamiliar conspecifics

We recorded the response to our simulated aggression in 16 instances (from 8 individuals), the first principal component explained 46% of the variance in the variables measured. Birds responded less aggressively to neighbors than stranger ($F_{1,5} = 30.4$, p = 0.003) after controlling for the distance from the roost center ($F_{1,6} = 20.05$, p = 0.004). Focal birds took longer to respond to challenges by neighbors and their responses were shorter and included less songs and harsh elements than their responses to strangers (Fig 1). This suggests that vocal distinctiveness allows acoustic discrimination to respond less aggressively to neighbors.

DISCUSION

We found that ocellated antbirds have individually distinctive vocalizations and can discriminate acoustically between neighbors and strangers. IR considers that an individual becomes discriminatively towards a specific individual based on its memory of prior encounters (Dale et al. 2001). We identify possible mechanisms underlying discrimination of conspecifics using acoustic signals in this species. First, receivers can learn individual distinctive song characteristics and associate these characteristics with specific information about the signaler. Receivers could also learn the signalers individually distinctive characteristics and associate these characteristics with inferred class-specific information of the signaler (Tibbetts & Dale 2007) i.e. neighbor or stranger. It is difficult to distinguish between the two possibilities because the only difference is the specificity of the information that the receiver learns from the signaler. In our study we didn't discriminate between the two mechanisms but we show that acoustic discrimination of conspecifics in these species occurs.

Reciprocal tolerance in dominance relations observed in *P. mcleannani* is a dynamic interaction that can function only if individual recognition is possible. In our study population prey intake decreases with the presence of other individuals (Chaves-

Campos et, al, in preparation), so there is a net benefit for the territory holder to selectively tolerate neighbors in his dominance area; therefore the capacity to discriminate between conspecifics is advantageous in this species. It is known that decreased aggression received from neighboring individuals favors individuals that broadcast information of its identity in their song (Temeles 1994). Ocellated antbirds have higher intake rates in the dominance area of nearest neighbors, probably due to reduced aggression from dominant males (Chaves-Campos et al. in preparation). Individuals should benefit from signaling their identity when producing contact loudsongs. The costs of signaling identity can inhibit the evolution of signals that broadcast the identity of the singer (Dale et al. 2001). Ocellated antbirds can pay the cost of aggression if they forage in the dominance areas of non-neighboring individuals and are identified as strangers. Therefore costs of broadcasting identity in the vocalizations depend on the probability to forage in a stranger territory. In our study population, ocellated antbirds fed outside the dominance area of their nearset neighbors 4 out of 10 days (Chaves-Campos et. al. in preparation), therefore it won't be a disadvantage to signal identity in the loudsong most of the time.

When we compare distinctiveness in vocalizations produced to contact their mate with distinctiveness in the aggressive vocalizations, it seems that pressure for individual recognition selects for distinctiveness in the vocalizations used to contact their mates. For contact loudsong was possible to identify correctly the singer in 90% of the time using only four acoustic features. In contrast to avhieve just 73% of correct classifications in aggressive vocalizations it was necessary 10 acoustic features. During vocal contests in aggressive interactions is expected that individuals try to produce vocalizations that reveal their fighting ability, therefore individuals should perform the most intimidating vocalizations they can. This will make that individuals with similar fighting ability have similar characteristics in the vocalizations. Meanwhile individuals should produce contact calls to be more easily recognized when arriving ant swarms; resulting in high individual distinctiveness in the vocalizations. When benefits of being recognized exists selection can act improving individuals distinctiveness by increasing phenotypic variability to make signalers memorably different (Dale et al. 2001).

Theoretical models have demonstrated that phenotypic variation needed for identity signals could evolve via negative frequency dependent selection (Dale et al. 2001), but our data does not allow us to conclude that this is the case in our species.

When we analyzed acoustic features separately, all measured features varied consistently among individuals. But the only acoustic features that remained stable through contexts were the frequency of the final and the lowest elements. Both acoustic features were in the set of variables that contributed the most to the distinctiveness of contact vocalizations, which supports the interpretation of their potential as identity cues. Both features have low frequencies; this means that the elements with lowest frequencies in the vocalizations were not affected by the motivational state of the producer. Its known that the lowest frequencies an individual can produce are determined in part by vocal tract length (Suthers 2004). This is a relatively fixed characteristic through an individual's adulthood. Motivational state independence and relatively fixed song characteristics through individuals life time are both traits predicted for identity signals (Dale et al. 2001) therefore these acoustic feature could be used as the recognition cues.

Our vocal distinctiveness results have relevance only if receivers can discriminate these levels of phenotypic variation, our playback experiment suggests that they can. Given that benefits attained by discriminating between conspecifics, could result in selection improving receiver's ability to discriminate finer degrees of phenotypic variation (Kanwisher & Yovel 2006) the possibility that these birds need even less information to discriminate between individuals' vocalizations is still an open question. We can conclude that vocal distinctiveness is enough to allow individual recognition in reciprocal tolerance relations of this obligate army ant-follower. This reciprocal tolerance relation makes it beneficial for individuals to recognize their neighbors and therefore have selected for neural specializations to discriminate between the vocalizations of different individuals. Also benefits from being recognized can produce positive selection for phenotypic variability and could explain some of the variation observed in the contact loudsong of this species.

REFERENCES

Bee, M. A. & Gerhardt, H. C. 2002 Individual voice recognition in a territorial frog (Rana catesbeiana). *Proceedings of the Royal Society of London Series B- Biological Sciences* **269**, 1443-1448.

Brennan, P. A. & Kendrick, K. M. 2006 Mammalian social odours: attraction and individual recognition. *Philosophical Transactions of the Royal Society B- Biological Sciences* **361**, 2061-2078.

Crowley, P. H., Provencher, L., Sloane, S., Dugatkin, L. A., Spohn, B., Rogers, L. & Alfieri, M. 1996 Evolving cooperation: The role of individual recognition. *Biosystems* **37**, 49-66.

Dale, J., Lank, D. B. & Reeve, H. K. 2001 Signaling individual identity versus quality: A model and case studies with ruffs, queleas, and house finches. *American Naturalist* **158**, 75-86.

Detto, T., Backwell, P. R. Y., Hemmi, J. M. & Zeil, J. 2006 Visually mediated species and neighbour recognition in fiddler crabs (Uca mjoebergi and Uca capricomis). *Proceedings of the Royal Society B-Biological Sciences* **273**, 1661-1666.

Dugatkin, L. A. & Earley, R. L. 2004 Individual recognition, dominance hierarchies and winner and loser effects. *Proceedings of the Royal Society of London Series B- Biological Sciences* **271**, 1537-1540.

Falls, J. B. 1982 Individual recognition by sounds in birds. In *Acoustic communication in birds*, vol. 2 (ed. M. E. H. Kroodsma D.E), pp. 237- 278. New York: Academic press.

Hooge, P. N. & Eichenlaub, B. 1997 *Animal movement extension to ARCVIEW, version 1.1. Alaska Science Center-Biological Science Office, U.S. Geological Survey, Anchorage, Alaska.*

Husak, J. F. & Fox, S. F. 2003 Adult male collared lizards, Crotaphytus collaris, increase aggression towards displaced neighbours. *Animal Behaviour* **65**, 391-396.

Kanwisher, N. & Yovel, G. 2006 The fusiform face area: a cortical region specialized for the perception of faces. *Philosophical Transactions of the Royal Society B-Biological Sciences* **361**, 2109-2128.

Karavanich, C. & Atema, J. 1998 Individual recognition and memory in lobster dominance. *Animal Behaviour* **56**, 1553-1560.

Kokko, H., Johnstone, R. A. & Clutton-Brock, T. H. 2001 The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 187-196.

Myrberg, A. A. & Riggio, R. J. 1985 Acoustically Mediated Individual Recognition by a Coral-Reef Fish (Pomacentrus-Partitus). *Animal Behaviour* **33**, 411-416.

Rendall, D., Rodman, P. S. & Emond, R. E. 1996 Vocal recognition of individuals and kin in free-ranging rhesus monkeys. *Animal Behaviour* **51**, 1007-1015.

Suthers, R. 2004 How birds sing and why it matters. In *Nature's Music. The Science of Bird Song* (ed. P. Marler, H. Slabbekorn), pp. 272-295
Elsevier Academic Press

Temeles, E. J. 1994 The role of neighbors in territorial systems: when are they dear enemies? *Animal Behaviour* **47**, 339-350.

Tibbetts, E. A. 2004 Complex social behaviour can select for variability in visual features: a case study in Polistes wasps. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 1955-1960.

Tibbetts, E. A. & Dale, J. 2007 Individual recognition: it is good to be different. *Trends in Ecology & Evolution* **22**, 529-537.

Trivers, R. L. 1971 Evolution of Reciprocal Altruism. *Quarterly Review of Biology* **46**, 35-&.

Vignal, C., Mathevon, N. & Mottin, S. 2008 Mate recognition by female zebra finch: Analysis of individuality in male call and first investigations on female decoding process. *Behavioural Processes* **77**, 191-198.

Willis, E. O. 1973 The behavior of Ocellated Antbirds. *Smithsonian Contributions to Zoology* **144**, 1-57.

Table 1. Discriminant function coefficients for the variables that contributed the most to distinctiveness in the contact vocalizations.

Variables	Function			
	1	2	3	4
F. last element	-.032	-.144	-1.648	1.324
F. middle element	.973	-.184	-.328	-.047
F. lowest element	.087	.185	2.103	-.378
Time to highest F	.196	.974	-.116	-.155

Table 2. ANOVA table for fixed and random effects derived from the generalized mixed models for each acoustic feature.

| Acoustic feature	Individual			Context		
	MS	F	p	MS	F	p
F. first element	0.28	4.9	<0.001	0.23	4.1	0.046
F. last element	0.32	3.40	<0.001	0.12	1.32	0.25
F. middle element	0.79	10.5	<0.001	1.39	18.4	<0.001
F. highest element	0.65	11.1	<0.001	0.99	16.97	<0.001
F. lowest element	0.3	5.41	<0.001	0.036	0.65	0.42
Max. F	0.67	8.82	<0.001	0.72	9.57	0.002
Bandwidth	0.32	4.22	<0.001	1.41	18.17	<0.001
Duration	4.6	5.58	<0.001	13.08	16.49	<0.001
Time to highest F	1.48	6.60	<0.001	0.92	4.09	0.046
Time to max F	0.67	8.82	<0.001	0.72	9.57	0.0026
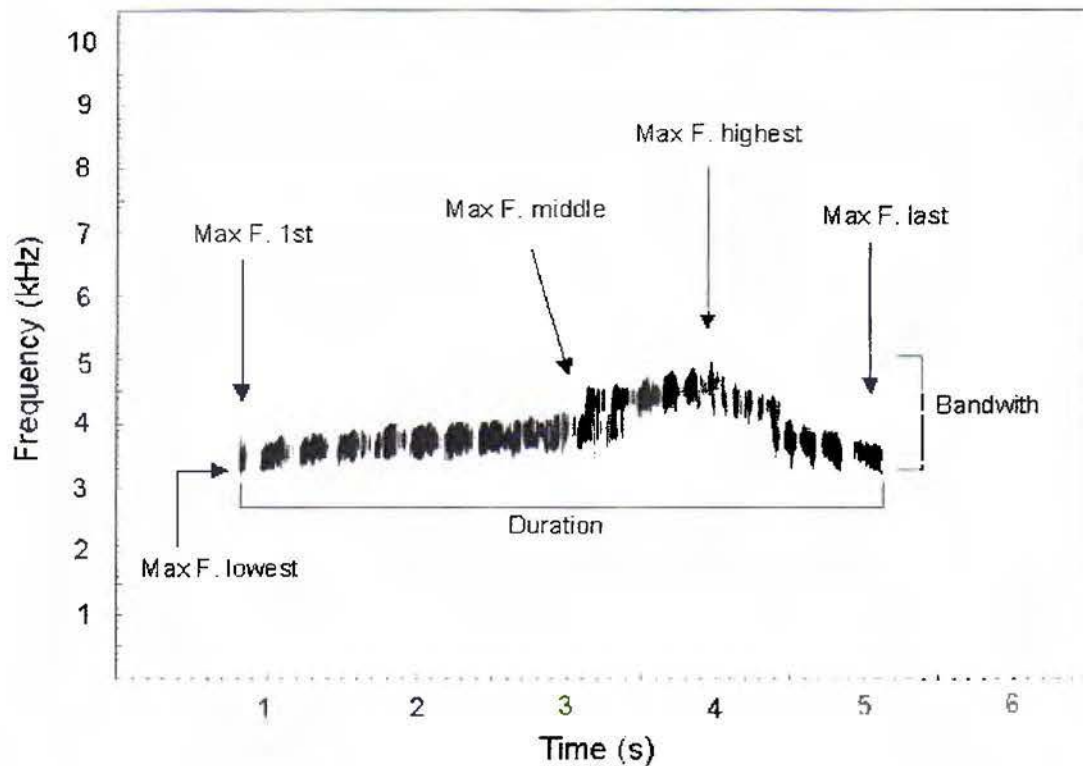
Figure 1. Spectrogram of the "loud song" of *P. mcleannani*. The horizontal axis shows time in seconds and the vertical axis presents frequency in KHz. The following measurements were taken using screen cursors: (1) maximum frequency of the loudsong (MaxF), (2) Bandwidth of the loudsong, (3) fundamental frequency of the first element (F 1st),(4) fundamental frequency of the element in the middle (F middle),(5)fundamental frequency of the last element (F. last), (6) fundamental frequency og the highest element (F. Highest), (7) fundamental frequency of the lowest element in the loudsong. (8) Duration of the loud song, (9) Time from the beginning of the vocalization until the highest pitched element, (10) Time from the beginning until the maximum frequency, (11) elements after the higher element in the vocalization.
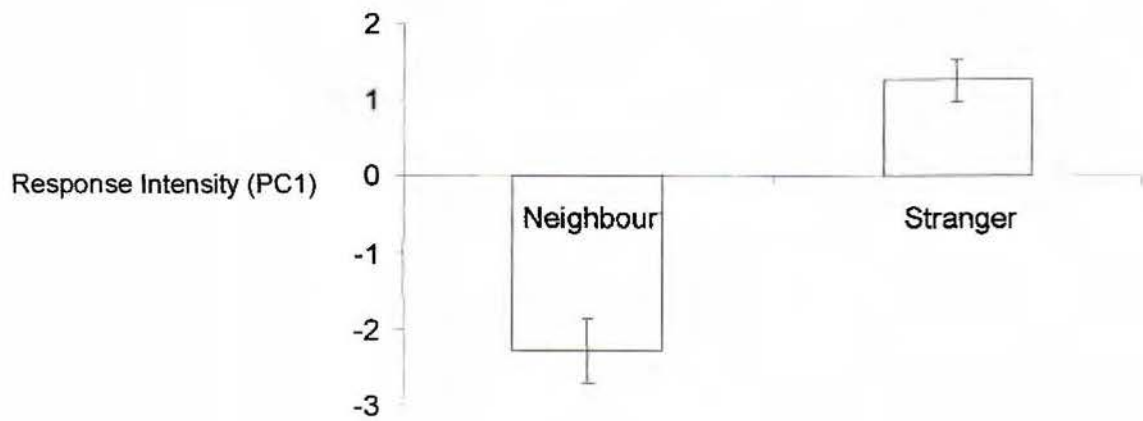
Figure 2. Reactions of 8 ocellated antbirds to simulated intrusions of neighbors and strangers. The response corresponds to the first component from a principal component analysis on the time to respond, response duration, vocalizations per response, and harsh elements per vocalization.