

2014

# Acoustic communication in thicket habitats: Vocal behaviour and vocal divergence in mesoamerican Melozone ground-sparrows

Luis Andres Sandoval Vargas  
*University of Windsor*

Follow this and additional works at: <https://scholar.uwindsor.ca/etd>

---

## Recommended Citation

Sandoval Vargas, Luis Andres, "Acoustic communication in thicket habitats: Vocal behaviour and vocal divergence in mesoamerican Melozone ground-sparrows" (2014). *Electronic Theses and Dissertations*. 5107.  
<https://scholar.uwindsor.ca/etd/5107>

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email ([scholarship@uwindsor.ca](mailto:scholarship@uwindsor.ca)) or by telephone at 519-253-3000ext. 3208.

**ACOUSTIC COMMUNICATION IN THICKET HABITATS:  
VOCAL BEHAVIOUR AND VOCAL DIVERGENCE IN MESOAMERICAN  
*MELOZONE* GROUND-SPARROWS**

by

LUIS ANDRÉS SANDOVAL VARGAS

A Dissertation  
submitted to the Faculty of Graduate Studies  
through the Department of Biological Science  
in Partial Fulfillment of the Requirements for  
the Degree of Doctor of Philosophy at the  
University of Windsor

Windsor, Ontario, Canada

© 2014 Luis Andrés Sandoval Vargas

Acoustic communication in thicket habitats: vocal behaviour and vocal divergence in  
Mesoamerican *Melospiza* ground-sparrows

by

Luis Andrés Sandoval Vargas

APPROVED BY:

---

T. Wright, External Examiner  
New Mexico State University

---

D. Heath  
GLIER

---

S. Doucet  
Department of Biological Sciences

---

O. Love  
Department of Biological Sciences

---

D. Mennill, Advisor  
Department of Biological Sciences

April 10, 2014

## **Declaration of Co-Authorship / Previous Publication**

### **I. Co-Authorship Declaration**

I declare that this thesis incorporates material that is the result of joint research, as follows: I am the sole author of the General Introduction, Chapter 1, and the General Discussion, Chapter 8. I am the principle author of the manuscripts that comprise the six data chapters: Chapters 2 through 7. All six data chapters are co-authored with my supervisor, Dr. Daniel Mennill. Chapters 1, 5, and 6 are co-authored with my field collaborator Carolina Méndez. Chapter 4 is co-authored with Dr. Torben Dabelsteen. Chapter 7 is co-authored with Dr. Stéphanie Doucet and Pierre-Paul Bitton. In all six instances, the key ideas are mine, and the experimental design, experimental execution, data analysis, interpretation, and writing were performed primarily by me. Dr. Daniel Mennill contributed to experimental design, data analysis, interpretation, and the writing and editing of the manuscripts, as well as providing financial and logistical support for the research described in all six data chapters. My others collaborators contributed to the experimental design, data interpretation, and/or writing and editing of the manuscripts.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from my co-authors to include the above material in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

Chapters 5 and 6 are published in the journals *Ethology* and *Animal Behaviour*, respectively, and are formatted for those journals. Chapters 2, 3, 4, and 7 are written in manuscript format for future submission.

## II. Declaration of Previous Publication

This thesis includes two original papers that have been published in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status
Chapter 5	Individual distinctiveness in the fine structural features and repertoire characteristics of the songs of White-eared Ground-sparrows	Published in Ethology
Chapter 6	Different vocal signals, but not prior experience, influence heterospecific from conspecific discrimination	Published in Animal Behaviour

I certify that I have obtained a written permission from the copyright owner(s) to include the above published material(s) in my thesis. I certify that the above material describes work completed during my registration as graduate student at the University of Windsor.

I declare that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my thesis.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

## Abstract

Acoustic communication is a critical component of social interactions in birds. There are relatively few quantitative studies of the vocal behaviour of tropical bird species, in spite of the rich avian biodiversity in the tropics and the extensive variety of vocalizations they produce. This lack of information inhibits our ability to understand the behaviour and ecology of tropical birds, and impairs our ability to perform comparative analyses from an evolutionary perspective. In this dissertation, I study the vocalizations of three species of tropical ground-sparrow: *Melozone biarcuata* (Prevost's Ground-sparrow), *Melozone kieneri* (Rusty-crowned Ground-sparrow), and *Melozone leucotis* (White-eared Ground-sparrow). I provide the first description of the vocalizations of each species, and demonstrate that all three ground-sparrows produce three main categories of vocalizations: calls, solo songs, and duets. I present results of a sound transmission experiment where I broadcast and re-recorded solo songs and duets through thicket habitats. I found that both vocalizations show similar patterns of degradation and attenuation with distance, suggesting that they facilitate communication with receivers at similar distances. I evaluate individual distinctiveness in the songs of male White-eared Ground-sparrows and the persistence of distinctive characteristics over time. I found that male White-eared Ground-sparrows sing individually distinctive songs. Uniquely, I found the frequency with which males sing different song types is also individually distinctive, and this feature varies little between recording sessions. I present results of a playback experiment to evaluate whether White-eared Ground-sparrows use calls, solo songs, and duets to discriminate conspecific from heterospecific competitors. I show that ground-sparrows display more intense responses to conspecific vocalizations than congeneric vocalizations, suggesting that they discriminate competitors from non-competitor species. Finally, I provide analyses of the morphology,

plumage patterns, colour reflectance, male solo songs, and calls of individuals from northern and southern subspecies of *Melospiza biarcuata*. My data show that the southern subspecies exhibits substantial phenotypic differences, on par with other subspecies complexes where species status has been recognized. I argue that *M. b. cabanisi* should be treated as a species separate from *M. biarcuata* (Prevost's Ground-Sparrow) and propose that it be called Cabanis' Ground-Sparrow (*Melospiza cabanisi*).

## Acknowledgements

I thank my supervisor Dr. Daniel J. Mennill for his support and assistance in conducting my dissertation research. His guidance taught me important skills as a successful investigator. I thank my Ph.D. committee members for their time and valuable comments during the development of my research: Dr. Stéphanie Doucet, Dr. Daniel Heath, and Dr. Oliver Love. I thank Dr. Tim Wright for agreeing to serve as the external examiner for my defence. I thank my collaborators on the projects in the data chapters of this dissertation who helped make these studies possible: Dr. Torben Dabelsteen, Dr. Stéphanie Doucet, and Pierre-Paul Bitton.

I thank Carolina Méndez, Olman Sandoval, Sharon Sandoval, Melania Fernández, and Sandra Gallo for assistance in the field, inside and outside of Costa Rica; their effort helping me to obtain the recordings and experimental data made this research possible. I thank Greg Budney and the Macaulay Library of Natural Sounds, Laboratorio de Bioacústica Universidad de Costa Rica, Jesse Fagan, and Knut Eisermann for sharing their recordings with me. I thank the Museo de Zoología Universidad de Costa Rica, Museo Nacional de Costa Rica, the Field Museum of Natural History, the University of Michigan Museum of Zoology, and the Musée National d'Histoire Naturelle of France for providing access to their skins collections. I thank Reserva Los Tarrales (Guatemala), Rancho Primavera (Mexico), Selva Negra (Nicaragua), and Estación Biológica Monteverde, Lankester Botanical Garden, and Bosquesito Leonel Oviedo (Costa Rica) for logistic support during field studies. I thank Ministerio del Ambiente y Energía (MINAE) and the Sistema Nacional de Áreas de Conservación (SINAC) for the permits to conduct my investigation in Costa Rica. I thank Gilbert Barrantes for help with obtaining permits to sample inside the Universidad de Costa Rica campus.

I thank Gregory Grether, Luis Ebensperger, four anonymous referees, Dave Wilson, Brendan Graham, and Roberto Sosa-López for comments that improved the quality of chapters 5 and 6. I also thank Tyne Baker, Sarah Baldo, Pierre-Paul Bitton, Adrianna Bruni, Alana Demko, Sarah Douglas, Brendan Graham, Kristina Hick, Julie Koloff, Kristin Kovach, Katrina Lukianchuck, Christie Macdonald, Dugan Maynard, Anneka Osmun, Nick Rehberg-Besler, Claire Sanders, Roberto Sosa-López, and David Wilson, for all your input on my dissertation research, but more importantly for your unconditional friendship.

This investigation was made possible by the scholarship support conferred by the Costa Rican Ministerio de Ciencia y Tecnología (MICIT) and Consejo Nacional para Investigaciones Científicas y Tecnológicas (CONICIT), the Government of Ontario, and the University of Windsor, as well a study grant through the Visiting Scholar program of the Field Museum of Natural History. This research was also made possible through grants to my supervisor from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Foundation for Innovation (CFI), the Government of Ontario, and the University of Windsor.

Finally, I am grateful for the support provided by my family members – my parents Olman and Xinia, my brother Daniel, and my sisters Yanin and Sharon – who always believed in me and supported my decisions. To Melania Fernández, who supported me through the good and the bad moments throughout this adventure, and who tolerated my absences while I pursued my research goals in Canada: thank you so much.

## Table of Contents

Declaration of Co-Authorship / Previous Publication .....	iii
Abstract.....	v
Acknowledgements.....	vii
List of Figures .....	xii
List of Figures .....	xii
List of Tables .....	xiv
Chapter 1: General Introduction .....	1
Introduction .....	2
Vocal behaviour in birds .....	5
Sound transmission and bird vocalizations .....	6
Geographic similarity and individual distinctiveness .....	7
Acoustic signal recognition .....	8
A multi-trait approach to understanding subspecies relationships.....	9
The <i>Melozone</i> genus .....	10
Behaviour and ecology of Mesoamerican ground-sparrows.....	12
Dissertation overview .....	13
References .....	16
Figures .....	22
Chapter 2: Description of the vocalizations and vocal behaviour of White-eared Ground-sparrows ( <i>Melozone leucotis</i> ) during the breeding season .....	23
Chapter summary .....	24
Introduction .....	26
Methods.....	27
Recording techniques .....	27
Vocalization measurements.....	28
Statistical analyses .....	30
Results .....	32
Calls .....	32
Solo Songs.....	33
Duets .....	34
Diel variation.....	36
Discussion .....	37
References .....	45
Figures .....	48
Chapter 3: A quantitative description of vocalizations and vocal behaviour of Rusty-crowned Ground-sparrows ( <i>Melozone kieneri</i> ).....	53
Chapter summary .....	54

Introduction .....	55
Methods.....	56
Field recordings .....	56
Vocal analysis.....	58
Statistical analysis .....	59
Results .....	60
Discussion .....	62
References .....	68
Figures .....	71
Chapter 4: Transmission characteristics of solo songs and duets in neotropical thicket habitat specialist bird .....	74
Chapter summary .....	75
Introduction .....	76
Methods.....	79
Study sites and territory measurements.....	79
Transmission playback stimuli .....	80
Transmission experiment.....	82
Sound analysis .....	83
Statistical analysis .....	84
Results .....	85
Vegetation characteristics .....	85
Degradation of complete solo songs and duets .....	86
Degradation of solo songs and duet elements.....	87
Background noise variation .....	88
Discussion .....	88
Conclusions.....	93
References .....	95
Tables.....	98
Figures .....	102
Chapter 5: Individual distinctiveness in the fine structural features and repertoire characteristics of the song of white-eared ground-sparrows.....	106
Chapter summary .....	107
Introduction .....	108
Methods.....	110
Song Classification and Measurements .....	112
Statistical Analysis.....	113
Results .....	117
Fine structure analysis .....	117
Repertoire-use similarity .....	118
Consistency over time.....	119
Discussion .....	120
References .....	124
Tables.....	129
Figures .....	130
Chapter 6: Different vocal signals, but not prior experience, influence heterospecific from conspecific discrimination.....	135

Chapter Summary .....	136
Introduction .....	137
Methods.....	141
Playback experiment .....	142
Response measures .....	145
Statistical analysis .....	147
Ethical note.....	149
Results .....	150
Discussion .....	152
References .....	156
Figures .....	160
Chapter 7: Analysis of plumage, morphology, and voice reveal species level differences between Prevost's Ground-sparrows subspecies .....	166
Chapter Summary .....	167
Introduction .....	168
Methods.....	170
Morphology .....	171
Plumage traits and spectrophotometry .....	171
Vocal analyses.....	174
Results .....	175
Morphology .....	175
Plumage patterns.....	176
Plumage colour .....	176
Voice .....	178
Discussion .....	179
References .....	184
Tables.....	188
Chapter 8: General Discussion .....	194
Significance .....	195
Suggestions for future research .....	196
Conclusion .....	199
References .....	201
Appendix.....	203
Vita Auctoris.....	215

## List of Figures

Chapter 1: General Introduction .....	1
<b>Figure 1.1.</b> Map showing the distribution of the three Mesoamerican <i>Melozone</i> ground-sparrows that are the focus of this dissertation .....	22
Chapter 2: Description of the vocalizations and vocal behaviour of White-eared Ground-sparrows ( <i>Melozone leucotis</i> ) during the breeding season .....	23
<b>Figure 2.1.</b> Sound spectrogram showing three examples of the two call types, <i>chip</i> and <i>tseet</i> , produced by both sexes of White-eared Ground-sparrows .....	48
<b>Figure 2.2.</b> Sound spectrogram showing six common male solo songs in White-eared Ground-sparrows at four study sites in Costa Rica. ....	49
<b>Figure 2.3.</b> Sound spectrogram showing three examples of complete duets (produced by male and female of the pair) and three incomplete duets (duet contributions produced by a single individual with no response from the partner) of White-eared Ground-sparrows. ....	50
<b>Figure 2.4.</b> Repertoire asymptote curve for 19 White-eared Ground-sparrows with 80 or more recorded songs, showing four males that reached an asymptote and one that did not. ....	51
<b>Figure 2.5.</b> Diel pattern of White-eared Ground-sparrow vocalizations. ....	52
Chapter 3: A quantitative description of vocalizations and vocal behaviour of Rusty-crowned Ground-sparrows ( <i>Melozone kieneri</i> ) .....	53
<b>Figure 3.1.</b> Sound spectrograms of the three main categories of vocalizations produced by Rusty-crowned Ground-sparrows .....	71
<b>Figure 3.2.</b> Repertoire asymptote curves for Rusty-crowned Ground-sparrows, showing six males that reached an asymptote .....	72
<b>Figure 3.3.</b> Diel variation in vocal output in Rusty-crowned Ground-sparrows according to calls, solo songs, and both vocalizations together .....	73
Chapter 4: Transmission characteristics of solo songs and duets in neotropical thicket habitat specialist bird .....	74
<b>Figure 4.1.</b> Spectrograms of the solo songs and duets, as well as the elements of solo songs and duets, of White-eared Ground-sparrows used in the transmission experiments .....	102
<b>Figure 4.2.</b> Variation in the four degradation measurements according to speaker and microphone heights used in the transmission experiments. ....	103
<b>Figure 4.3.</b> Variation in the four degradation measurements according to sounds for complete solo songs and duets, and solo song and duet elements used in the transmission experiments. ....	104
<b>Figure 4.4.</b> Second order interactions between distance and speaker and microphone heights (black, 0.4 m; white, 2.2 m) for complete solo songs and duets. ....	105
Chapter 5: Individual distinctiveness in the fine structural features and repertoire characteristics of the song of white-eared ground-sparrows .....	106
<b>Figure 5.1.</b> Map showing four populations of white-eared ground-sparrows in Costa Rica where male songs were recorded for analyses of individual distinctiveness .....	130

<b>Figure 5.2.</b> Sound spectrograms representing six common solo song types, with examples from three different male white-eared ground-sparrows for each type. ....	131
<b>Figure 5.3.</b> Sound spectrogram of a typical white-eared ground-sparrow song, showing the 17 fine structural features we measured.....	132
<b>Figure 5.4.</b> Comparison between male solo song repertoire-use similarity in white-eared ground-sparrows, using the Morisita index of similarity, comparing shared song types and the frequency of utilization of each song type (N = 38).....	133
<b>Figure 5.5.</b> Comparison between recording sessions of male solo song repertoire in white-eared ground-sparrows, using the Morisita index of similarity, comparing shared song types and the frequency of utilization of each song type within males (N = 13).. ....	134
 Chapter 6: Different vocal signals, but not prior experience, influence heterospecific from conspecific discrimination.....	
	135
<b>Figure 6.1.</b> (Previous page) Sound spectrograms of three types of vocalizations used in the playback experiment to study species recognition in white-eared ground-sparrow.....	161
<b>Figure 6.2.</b> Schematic representation of the timing of playback trials delivered to white-eared ground-sparrows.. ....	162
<b>Figure 6.3.</b> Comparison of responses displayed by white-eared ground-sparrow pairs to playback of three types of vocalizations .....	163
<b>Figure 6.4.</b> Comparison of responses displayed by white-eared ground-sparrow pairs to playback of three types of vocalizations .. ....	164
<b>Figure 6.5.</b> Vocal responses of white-eared ground-sparrow pairs to playback of three types of vocalizations.....	165
 Chapter 7: Analysis of plumage, morphology, and voice reveal species level differences between Prevost's Ground-sparrows subspecies .....	
	166
<b>Figure 7.1.</b> Distribution of the <i>Melospiza biarcuata</i> subspecies from Mexico to Costa Rica. ....	190
<b>Figure 7.2.</b> Plumage colour and pattern differences between <i>M. b. biarcuata</i> (left) in <i>M. b. cabanisi</i> (right). ....	191
<b>Figure 7.3.</b> Average reflectance spectra for ten body regions measured in 11 <i>M. b. biarcuata</i> , 9 <i>M. b. hartwegi</i> , and 11 <i>M. b. cabanisi</i> .....	192
<b>Figure 7.4.</b> Sound spectrograms of calls (a, b), male solo songs (c - f), and duets (g, h) of <i>M. b. biarcuata</i> (left) and <i>M. b. cabanisi</i> (right). ....	193

## List of Tables

Chapter 4: Transmission characteristics of solo songs and duets in neotropical thicket habitat specialist bird.....	74
<b>Table 4.1.</b> Main effects and two-factor interactions in the general linear models comparing the complete solo songs versus complete duets for each attenuation and degradation measurement. ....	98
<b>Table 4.2.</b> Variation in the four degradation measurements (average $\pm$ SE) according to distance analyzed in the transmission experiment of complete solo song and duets, and solo song and duet elements. ....	99
<b>Table 4.3.</b> Main effects and two-factor interactions in the general linear models comparing the solo song elements versus duet elements for each attenuation measurement. ....	100
<b>Table 4.4.</b> Main effects and two-factor interactions in the two general linear models comparing the background noise across complete solo song and duets; and elements of solo songs and duets.....	101
Chapter 5: Individual distinctiveness in the fine structural features and repertoire characteristics of the song of white-eared ground-sparrows.....	106
<b>Table 5.1.</b> Results of six discriminant function analyses (DFA) used to evaluate individual distinctiveness in male white-eared ground-sparrow songs. ....	129
Chapter 7: Analysis of plumage, morphology, and voice reveal species level differences between Prevost’s Ground-sparrows subspecies .....	166
<b>Table 7.1</b> Mean ( $\pm$ SE) morphometric measurements by sex in three <i>Melospiza biarcuata</i> subspecies.. ....	188
<b>Table 7.2</b> Mean ( $\pm$ SE) values of male solo song and call fine acoustic measurements by sex and <i>Melospiza biarcuata</i> subspecies. ....	189
Appendix.....	203
<b>Table S5.1:</b> Fine-scale song measurements for song type 1 shared between White-eared Ground-sparrow males; coefficients of variation between males (CVb) and within males (CVw), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. ....	204
<b>Table S5.2:</b> Fine-scale song measurements for song type 2 shared between White-eared Ground-sparrow males; coefficients of variation between males (CVb) and within males (CVw), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement.. ....	205
<b>Table S5.3:</b> Fine-scale song measurements for song type 3 shared between White-eared Ground-sparrow males; coefficients of variation between males (CVb) and within males (CVw), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. ....	206
<b>Table S5.4:</b> Fine-scale song measurements for song type 18 shared between White-eared Ground-sparrow males; coefficients of variation between males (CVb) and within males (CVw), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. ....	207

<b>Table S5.5:</b> Fine-scale song measurements for song type 20 shared between White-eared Ground-sparrow males; coefficients of variation between males (CVb) and within males (CVw), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. ....	208
<b>Table S5.6:</b> Fine-scale song measurements for song type 24 shared between White-eared Ground-sparrow males; coefficients of variation between males (CVb) and within males (CVw), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. ....	209
<b>Table S6.1.</b> Playback responses of white-eared ground sparrows (average $\pm$ SE) separated by the type and the species of playback stimulus, for both the sympatric and allopatric populations. ....	210
<b>Table S6.2.</b> Acoustic features of the calls produced by white-eared ground sparrows (average $\pm$ SE) separated by the type and the species of playback stimulus, for both the sympatric and allopatric populations. ....	211
<b>Table S6.3.</b> Acoustic features of the calls and duets produced by white-eared ground sparrows (average $\pm$ SE) separated by the type and the species of playback stimulus, for both the sympatric and allopatric populations. ....	212
<b>Table S7.1.</b> List of skins used in this study that were measured at Museo de Zoología Universidad de Costa Rica (UCR), Museo Nacional de Costa Rica (MNCR), the Field Museum of Natural History (FMNH), the University of Michigan Museum of Zoology (MZUM), and the Muséum National d'Histoire Naturelle (MNHN). ....	213
<b>Table S7.2.</b> List of recordings used in this study that were obtained from Laboratorio de Bioacústica Universidad de Costa Rica (UCR), the Macaulay Library of Natural Sounds Cornell Laboratory of Ornithology (ML), the private collection of Jesse Fagan (JF), and the private collection of Knut Eisermann (KE).....	214

## **Chapter 1**

### **General Introduction**

## **Introduction**

Evolution shapes animal vocalizations through natural selection, sexual selection, and drift (Arak and Enquist 1993). These processes may generate divergent vocal signal characteristics among species (Bertelli and Tubaro 2002, Seddon 2005, Tobias and Seddon 2009), or among populations of the same species (Dingle et al. 2008), thereby influencing speciation (West-Eberhard 1983). In addition, these processes may influence the efficiency of information transfer from signaler to receiver through the environment (Morton 1975, Hansen 1979). It remains unclear which process (natural, sexual selection, or drift) is the principal process that influences the divergence of animal vocalizations, or whether these processes share equal importance in shaping animal sound (Slabbekoorn and Smith 2002, Seddon 2005, Dingle et al. 2008). The effects of natural selection (as it relates to morphological or ecological factors, such as body size or habitat-based influences on vocalizations) or drift on vocal divergence are still poorly studied (Slabbekoorn and Smith 2002), whereas processes related to sexual selection have received more attention (i.e. female mate choice and male-male competition; reviewed in Collins 2004).

Two important hypotheses have been proposed for analyzing acoustic signal adaptation (vocal characteristics that positively influence fitness) in relation to habitat and morphology: the Acoustic Adaptation Hypothesis and the Morphological Hypothesis. The Acoustic Adaptation Hypothesis (Morton 1975, Hansen 1979) states that environment shapes the evolution of animal signals by selecting for signals that effectively transmit information between signalers and receivers, so that acoustic characteristics of animal vocalizations are adapted to the habitat where they are typically transmitted. As a consequence, natural selection can generate convergent signal characteristics between species that inhabit areas with similar vegetation characteristics, especially if habitat characteristics influence the design of acoustic signals (Wiley

1991, Boncoraglio and Saino 2007, Ey and Fisher 2009). The Morphological Hypothesis states that morphology of the syrinx, beak, and other physical structures influences the characteristics of animal signals (Nowicki et al 1992). As a consequence, vocal similarity is more probable in closely related species (Podos 2001, Bertelli and Tubaro 2002, Seddon 2005, Jiggins et al. 2006), because they share similar body features such as body and bill morphology (Qvarnström et al. 2006, Tobias and Seddon 2009, Seddon and Tobias 2010). Numerous studies have found support for both the Acoustic Adaptation Hypothesis (including: Wiley 1991, Tubaro and Segura 1994, Patten 2004) and the Morphological Hypothesis (including: Podos 2001, Bertelli and Tubaro 2002, Seddon 2005). It therefore appears that both hypotheses are important for understanding the evolution of animal vocalizations.

The social behaviour of different animal species may also affect the evolution of their vocal signals (Forrest 1994). If receivers are usually far away from signallers, as is common in species that live at low densities or have large territories, then signals need to propagate over long distances with little degradation (distortion of acoustic characteristics of the vocal signals) and attenuation (loss of sound amplitude related to transmission distance). In such cases, vocal evolution will be heavily influenced by acoustic adaptation to transmit with maximal efficiency through the environment (Brown and Handford 2000). In contrast, when signallers and receivers are usually found in close proximity, as is common in animals that live in social groups or have small territories, this selective pressure may be relaxed (Dabelsteen 2005). In these cases, signals experience less degradation and attenuation, even in spite of living in areas with dense vegetation, because signalers and receivers are near to one another. Consequently, the social behaviour of animals and the spacing between individuals have a strong influence on the role that the habitat plays in shaping animal vocal signals.

Although all of these factors can affect vocal signal divergence in different ways, few studies have attempted to analyze simultaneously the effects of social, ecological, and morphological factors on several vocalization types (e.g., calls, solo songs, and duets). In this dissertation, I analyze the influence of vegetation, spatial distribution, and social competition on the vocal signals of Mesoamerican ground-sparrows (*Melospiza* sp.). These songbirds are suitable models for such an investigation because they live in dense thicket habitats (Stiles and Skutch 1989, Howell and Webb 2004, Rising 2011, Sandoval and Mennill 2012) where vocal signals are very important components of social and sexual interactions (Sandoval and Barrantes 2012). My main goal in studying this group of birds is to provide a better understanding of the main forces affecting the divergence of different types of vocal signals. By describing vocal signals in the ground-sparrow species in chapters 2 and 3, I seek to provide background information for future comparative studies between taxa and experimental questions about vocal signal function and evolution. By experimentally analyzing the effect of habitat on solo song and duet transmission in chapter 4, I seek to provide a better understanding of sound transmission in two different vocal signals. By analyzing the individual distinctiveness and the temporal and spatial variability among individuals' repertoires in chapter 5, I seek to understand individual-level variation, and variation between populations. By analyzing birds' responses to conspecific and congeneric vocal signals in chapter 6, I seek to provide a better understanding of how animals perceive acoustic differences. By analyzing the similarity between subspecies' vocal signals, morphology, and plumage patterns in chapter 7, I seek to provide a better understanding of the taxonomic relationships within ground-sparrow species. In this introductory chapter, I introduce each of the major topics addressed in this dissertation and I briefly summarize what is known about the natural history of my study species.

### **Vocal behaviour in birds**

The complete library of vocal signals produced by a species or an individual is called a vocal repertoire (Catchpole and Slater 2008). In Oscine songbirds, the suborder of birds where ground-sparrows are classified, some vocal signals may be inherited genetically (e.g., calls), while others may be learned culturally (e.g., solo songs and possibly duets; Kroodsma 2004, Marler 2004, Beecher and Brenowitz 2005). For example, male vocalizations used to attract females may diverge at faster rates than vocalizations that indicate food or alarm in response to predators (Andersson 1994, Marler 2004). This occurs because the selection process associated with more rapid divergence is often female mate choice, driving strong directional selection (Andersson 1994). Another reason for faster divergence in learned vocalizations is that cultural inheritance is imperfect; significant changes can occur in every generation as individuals introduce small errors while they learn the sounds of adults (Beecher and Brenowitz 2005, Wright et al. 2008). Meanwhile, less divergent vocalizations appear to be related to behaviours that experience stabilizing selection, including naturally-selected vocalizations that are important in food-finding or alarm contexts (Marler 2004). Descriptive vocal studies of vocal repertoires among related bird species are critical for helping us to understand the variation in signals between species, and to facilitate comparative studies between both song (a sexually-selected signal) and non-song vocalizations (subject to different types of selection).

### **Sound transmission and bird vocalizations**

Several studies have revealed that animals produce particular types of vocalizations, and engage in particular vocal behaviours, that maximize the transmission distance of their vocal signals (Krams 2001, Mathevon et al. 2005, Barker and Mennill 2009, Barker et al. 2009). These vocalizations are referred to as long-distance signals or public signals (Dabelsteen 2005). Other opportunistic receivers can also access the information encoded in the vocalizations, including competitors, parasites, and predators (McGregor and Peake 2000). Efficient transmission of long-distance signals is important in sexual and social interactions because it allows contact between mates and other conspecifics and may help in territory defence (Boncoraglio and Saino 2007). The influence of local vegetation and ambient noise on sound propagation also depends on the distance between the signaller and receiver (Forrest 1994, Barker et al. 2009). If the vocalization must travel large distances to reach the potential receiver, it will experience more degradation (Wiley 1991).

By comparing the transmission and degradation characteristics of vocal signals, and by including both long-distance signals as well as short-distance signals, we may gain a deeper appreciation for the influence of habitat on animal vocal signals. For animals that live in habitats where sound propagation is challenging (e.g., close to noisy rivers and roads, or habitats with dense vegetation; Slabbekoorn and Smith 2002, Redondo et al 2013), acoustic adaptation can lead to vocalizations with structural characteristics that enhance vocal transmission (McGregor and Krebs 1984, Wiley 1991). Therefore, transmission studies of vocalizations by bird species that live in such habitats (e.g., thickets habitats) may provide significant insight into the influence of habitat on animal vocal signals.

### **Geographic similarity and individual distinctiveness**

Geographic variation in vocalizations is well documented across a wide variety of taxa including amphibians (Gerhardt 1994), mammals (e.g., Conner 1982, Thomas and Stirling 1983, Davidson and Wilkinson 2002), and birds (Kroodsma 2004). Isolation, habitat differences, and drift between isolated groups have all been shown to give rise to population-level variation within species (reviewed in Catchpole and Slater 2008, Podos and Warren 2007), resulting in more similar vocal characteristics among nearby animals (Baker and Cunningham 1985, Salinas-Melgoza and Wright 2012). Many studies of geographic variation in avian vocalizations have found such patterns, with particularly strong divergence of male solo songs in bird species that exhibit vocal learning (reviewed in Kroodsma 2004). In vocal learning species, divergence in vocalizations does not necessarily follow patterns of genetic variation. In White-crowned Sparrows (*Zonotrichia leucophrys*), for example, groups of birds with the same dialect do not share genetic similarities (MacDougall-Shackleton and MacDougall-Shackleton 2001). In Yellow-naped Parrots (*Amazona auropalliata*), the two dialects observed in Costa Rica are not related with genetic variation (Wright and Wilkinson 2001).

Unique features between the songs of different individuals facilitate individual recognition (Barnard and Burk 1979, Dale et al. 2001, Tibbetts and Dale 2007), signaling individual quality (Christie et al. 2004, Brumm 2009, Byers et al. 2010), and group or geographic origin (Sewall 2009, 2011). Signals used for individual recognition vary between species (Ptacek 2000, Ord and Stamps 2009, Grether 2011, Ord et al. 2011), but individually-distinctive acoustic features are known to occur in the vocalizations of amphibians (e.g. Bee et al. 2001, Feng et al. 2009, Gasser et al. 2009), birds (e.g. Harris and Lemon 1972, Lovell and Lein 2004, Sandoval and Escalante 2011), and mammals (e.g. Dallmann and Geissmann 2001, Blumstein and Munos 2005,

Vannoni and McElligott 2007). Consistency of these signals over time is a requirement to allow receivers to recognize the individual that produced the signal (Ellis 2008). However, similarities between acoustic characteristics of individual songs (e.g., temporal characteristics, frequency characteristics) and repertoires (number of song types) may change over time due to the occurrence of open-ended learning (song learning each year; Vargas-Castro et al. 2012), changes in signaller physiology and morphology (Nottebohm et al. 1987, Gil and Gahr 2002), or changes in the habitat where the song is transmitted (Forrest 1994, Slabbekoorn et al. 2002). Quantification of geographic and temporal variation of the acoustic characteristics and repertoire of vocalizations offers insights into the vocalization learning process.

### **Acoustic signal recognition**

Vocalizations in birds are used for multiple purposes including mate attraction, resource defence (defence of breeding partners, territories), and species recognition (Catchpole and Slater 2008). Therefore, it is important that these signals are correctly recognized by the receiver. In some cases, vocal similarity between species can produce misidentifications (Qvarnström et al. 2006, Tobias and Seddon 2009, Seddon and Tobias 2010), resulting in interspecific aggression and hybridization (Baker and Boylan 1999, Qvarnström et al. 2006, Tobias and Seddon 2009). Understanding the mechanisms that permit conspecific recognition is essential for understanding the evolution of vocal signals between species.

For territorial birds that inhabit sites with dense vegetation, vocal signals may be the optimal form of communication for interacting with conspecific individuals because visual signals will transmit only short distances. Similarity in vocalizations (e.g., calls, solo songs, and duets) between species may result in species misidentification when multiple species are present in the

same area and visual cues are limited. For example, studies in *Ficedula* flycatchers (Qvarnström et al. 2006) and *Hypocnemis* antbirds (Tobias and Seddon 2009, Seddon and Tobias 2010) revealed that vocal similarity produced direct interactions between sympatric species. The response to similar vocal stimuli, however, could be the result of a learned or innate behaviour. Therefore, to understand how similar vocal stimuli are recognized by the animals, it is worthwhile to conduct comparisons between experienced and non experienced populations with respect to the stimulus of interest.

### **A multi-trait approach to understanding subspecies relationships**

Given that one of the most common units of conservation is the species, it is important to explicitly and clearly define what constitutes a species (Garnet and Christidis 2007). Multiple definitions and approaches for identifying species exist (De Queiroz 2005, Cadena and Cuervo 2010), but many ambiguities remain. For example, the Morphospecies Concept states that a species is a group of organisms that share identical morphological characters. The Biological Species Concept states that a species is a group of organisms that can interbreed freely (Mayr 1969). The Phylogenetic Species Concept states that a species is a group of organism that share the same ancestry (Cracraft 1989). The Evolutionary Species Concept states that a species is a group of organisms that share the same evolutionary trajectory (Wiley 1978, Wiley and Mayden 2000, Peterson 2006, 2007). Problems with these species definitions arise when we compare allopatric populations of the same organism or different populations of similar organisms showing small variation in one or several traits (De Queiroz 2005, Cadena and Cuervo 2010). Another problem with species definitions occurs when two different organisms interbreed to produce hybrids (De Queiroz 2005, Cadena and Cuervo 2010).

In the tropics, species definitions are more challenging because many species are poorly studied and there is a lack of genetic data for many taxa. A multi-trait approach has recently been proposed for taxonomic studies when genetic data are lacking (Tobias et al. 2010, Cadena and Cuervo 2010). A multi-trait approach involves evaluating morphological, behavioural, and ecological traits simultaneously, and if two organisms differ in several uncorrelated traits, it is probable that both forms will be recognized as different species (Tobias et al. 2010, Cadena and Cuervo 2010). This approach has been used effectively in studies of a brush-finch (the *Arremon torquatus* complex; Cadena and Cuervo 2010) and the Long-tailed Antbird (*Drymophila caudata*; Isler et al. 2012). In the brush-finch, 8 of the 14 recognized subspecies currently classified within *A. torquatus* are sufficiently different in terms of vocalizations, plumage patterns, ecological niches, and genetics to be recognized as different species (Cadena and Cuervo 2010). In the antbird, vocalizations, niche ecology, and genetic patterns were sufficiently different to separate 8 subspecies into 4 species (Isler et al. 2012).

### **The *Melozone* genus**

Ground-sparrows (genus: *Melozone*) are small granivorous birds, with body sizes that range from 15 to 24 cm in length, weighing 24.8 to 61.2 g (Rising 2011). They are found from southwestern Oregon to central Costa Rica, primarily in habitats along the Pacific coast, ranging from sea level to 2950 m (Stiles and Skutch 1989, Tweit and Fish 1994, Howell and Webb 1995, Johnson and Haight 1996, Benedict et al. 2011). Their plumage colours vary from brown to grey, typically with whitish breasts and reddish undertail coverts (Rising 2011).

Historically, the *Melozone* genus included only three Mesoamerican species, but the taxonomy has changed recently as a result of genetic studies (DaCosta et al. 2009, Chesser et al.

2010). Four towhee species formerly recognized as part of the *Pipilo* genus (Tweit and Fish 1994, Howell and Webb 1995, Johnson and Haight 1996, Benedict et al. 2011) are now classified as *Melozone*. Consequently, the genus *Melozone* has now increased from three species to seven (Chesser et al. 2010). The four recently added *Melozone* species — Abert's Towhee (*Melozone aberti*), California Towhee (*M. crissalis*), Canyon Towhee (*M. fusca*), and White-throated Towhee (*M. albicollis*) — are distributed from Pacific and central Mexico to the central and West coast of the United States of America (Tweit and Fish 1994, Howell and Webb 1995, Johnson and Haight 1996, Benedict et al. 2011). All four species have brown plumage and lack conspicuous plumage patterns, which sometimes results in visual misidentification. For example, the similarity in plumage between California and Canyon Towhees meant that these species were previously considered a single species (Benedict et al. 2011). The natural history, behaviour, and ecology of three of the four recently-added species is well-studied (see Tweit and Fish 1994, Howell and Webb 1995, Johnson and Haight 1996, Benedict et al. 2011, Rising 2011), whereas more research is needed on White-throated Towhees (*M. albicollis*).

The second group within this genus — the Mesoamerican ground-sparrows (Stiles and Skutch 1989, Howell and Webb 1995, AOU 1998, Rising 2011) — are the species studied in this dissertation: Prevost's Ground-sparrows (*Melozone biarcuata*), Rusty-crowned Ground-sparrows (*M. kieneri*), and White-eared Ground-sparrows (*M. leucotis*). These three tropical species are distributed from western and central Mexico to the centre of Costa Rica (Figure 1.1). All three species show conspicuous plumage patterns in the head and breast, including black, white, yellow, or orange spots (Figure 1.1; Stiles and Skutch 1989, Howell and Webb 1995). In contrast with the more northerly species of *Melozone*, the natural history, behaviour, and ecology of

these three tropical *Melospiza* species is limited to anecdotal descriptions (Stiles and Skutch 1989, Howell and Webb 1995, Rising 2011).

### **Behaviour and ecology of Mesoamerican ground-sparrows**

There are few descriptions of the behaviour and ecology of Mesoamerican ground-sparrows. Previous descriptions suggest that pairs defend territories year round in young secondary vegetation, forest edges, shaded coffee plantations, and thicket vegetation (Stiles and Skutch 1989, Howell and Webb 1995, AOU 1998). Analyses that I conducted concurrently with this dissertation (Sandoval and Mennill 2013) found that the sexes are monochromatic to the human eye, but morphologically different for all three species. Analyses of 82 White-eared Ground-sparrows (*M. leucotis*) revealed that males are larger than females for only a single morphological trait: wing length (Sandoval and Mennill 2013). In contrast, analyses of 56 Prevost's Ground-sparrows (*M. biarcuata*) and 32 Rusty-crowned Ground-sparrows (*M. kieneri*), revealed that males are larger than females for multiple morphological traits including beak size, tarsus, tail, and wing length in *M. biarcuata*; and tarsus, wing, and culmen length in *M. kieneri* (Sandoval and Mennill 2013).

Anecdotally, vocalizations of ground-sparrows species are classified into three main types: calls, male solo songs, and duets (Stiles and Skutch 1989, Howell and Webb 1995, Rising 2011). Calls are high-pitched vocalizations that appear to be produced in multiple contexts, and are thought to vary in rate when birds are foraging or when a predator is close (Marler 2004). The male solo song has been suggested to be the most variable vocalization among and within species. The general structure of this vocalization has been described as beginning with a high-pitched call, followed by a trill and/or modulated whistle, and ending with a trill (Stiles and

Skutch 1989). Duets have been described as a series of sharp and thin calls (Stiles and Skutch 1989, Howell and Webb 1995). The vocalizations of all three tropical *Melospiza* species have never been described in any quantitative bioacoustic detail.

### **Dissertation overview**

In this dissertation I describe the vocal repertoire and vocal behaviour of Mesoamerican ground-sparrow species, I evaluate the effect of habitat on transmission properties of different vocalizations, and I describe individual distinctiveness and the effect of geographic distribution and temporal variation on song repertoire similarity. I evaluate factors promoting differential responses to conspecific and congeneric vocal stimuli using playback, and I provide information about the taxonomic status of some of the Mesoamerican ground-sparrows using a multi-trait approach using morphological measurements, acoustic recordings, and plumage patterning and reflectance.

In chapters 2 and 3, I provide the first description of the structural and temporal characteristics of the vocalizations of two Mesoamerican ground-sparrow species: White-eared Ground-sparrows (*M. leucotis*) and Rusty-crowned Ground-sparrows (*M. kieneri*). In chapter 2, I provide a description of the vocal repertoire and pattern of diel variation in vocal behaviour in White-eared Ground-sparrows, including detailed measurements of the acoustic structure of vocalizations, an analysis of the syntax of male solo song, and information about context of use of vocalizations. In chapter 3, I provide a description of the vocal repertoire and the diel pattern of variation in Rusty-crowned Ground-sparrows, including detailed measurements of the acoustic structure of vocalizations, an analysis of the syntax of male solo song, information

about context of use of vocalizations, and analysis of similarity in solo song repertoires between neighbours.

In chapter 4, I analyze the transmission properties of different types of vocalizations within the territories of White-eared Ground-sparrows. I use a sound transmission experiment to compare if solo songs and duets produced by this species degrade similarly (at similar distances and heights) when transmitting through their territories. This comparison evaluates whether both vocalization types facilitate communicate with receivers at similar distances and positions.

In chapter 5, I evaluate individual distinctiveness in the acoustic characteristics of male solo songs and I compare song repertoires between individuals over time in four populations of White-eared Ground-sparrows. I also evaluate the similarity between solo song repertoires between neighbouring males. This chapter was recently published in the journal *Ethology*.

In chapter 6, I evaluate how territorial pairs of White-eared Ground-sparrows discriminate between vocal signals – including calls, solo songs, and duets – of conspecific and congeneric competitor species. I evaluate the effect of previous experience on the response to these vocal signal types, using populations that are allopatric and sympatric with the congeneric species. This chapter was recently published in the journal *Animal Behaviour*.

In chapter 7, I use a multi-trait approach to evaluate the relationship between Prevost's Ground-sparrow (*M. biarcuata*) subspecies. Firstly, I compare the morphology between subspecies by sex. Secondly, I compare plumage spectral characteristics of ten body regions per subspecies, using a visual model to take into account the perception of visual signals by receivers. Finally, I compare fine acoustic characteristic of calls, solo songs, and duets between subspecies.

My dissertation uses an integrative approach, combining observations from animals in the field, museum studies, and experiments to describe animal behaviour and ecology, and to test hypotheses on the causes of vocal divergence among species. I also evaluate how multiple phenotypic traits can help to understand taxonomic relationships between closely related organisms. This research will expand our understanding of the influence of vegetation, spatial distribution, and social competition on the divergence of different vocal signals within birds, while also expanding our understanding of the vocalizations of a poorly-studied group of animals, the Mesoamerican ground-sparrows. Finally, in this dissertation I discuss how the different vocal characteristics of my study animals could be adaptations, although I do not directly test the relationship between vocal characteristics and survival or reproductive success, or the inheritance of the characteristics that I describe.

## References

- Andersson M. 1994. Sexual selection. Princeton University Press, New Jersey.
- American Ornithologists' Union (AOU) (1998) Check list of North American birds, 7th edition. American Ornithologists' Union, Washington.
- Arak A, Enquist M. 1993. Hidden preferences and the evolution of signals. *Phil. Trans. R. Soc. Lond. B* 340:207-213.
- Baker MC, Boylan JT. 1999. Singing behavior, mating associations and reproductive success in a population of hybridizing Lazuli and Indigo Buntings. *Condor* 101:493-504.
- Baptista LF. 1996. Nature and its nurturing in avian vocal development. In *Ecology and evolution of acoustic communication in birds* (Kroodsma DE, Miller EH, eds). Cornell University Press, Ithaca. Pp. 39-60.
- Baker MC, Cunningham MA. 1985. The biology of birdsong dialects. *Behav. Brain Sci.* 8:85-133.
- Barker NK, Mennill DJ. 2009. Song perch height in Rufous-and-White Wrens: does behaviour enhance effective communication in a tropical forest? *Ethology* 115:897-904.
- Barker NK, Dabelsteen T, Mennill DJ. 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.
- Barnard CJ, Burk T. 1979. Dominance hierarchies and the evolution of "individual recognition". *J. Theor. Biol.* 81:65-73.
- Bee MA, Kozich CE, Blackwell KJ, Gerhardt HC. 2001. Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. *Ethology* 107:65-84.
- Beecher M, Brenowitz E. 2005. Functional aspects of song learning in songbirds. *Trend. Ecol. Evol.* 20:143-149.
- Benedict L, Kunzmann MR, Ellison K, Purcell KL, Johnson RR, Haight LT. 2011. California Towhee (*Melospiza crissalis*). In: *The birds of North America Online* (Poole A, ed). Cornell Lab of Ornithology, Ithaca.
- Bertelli S, Tubaro P. 2002. Body mass and habitat correlates of song structure in a primitive group of birds. *Biol. J. Linn. Soc.* 77:423-430.
- Blumstein DT, Munos O. 2005. Individual, age and sex-specific information is contained in yellow-bellied marmot alarm calls. *Anim. Behav.* 69:353-361.
- 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.
- Brown TJ, Handford P. 2000. Sound desing for vocalizations: quality in the woods, consistency in the fields. *Condor* 102:81-92.
- Brumm H. 2009. Song amplitude and body size in birds. *Behav. Ecol. Sociobiol.* 63:1157-1165.
- Byers J, Hebets E, Podos J. 2010. Female mate choice based upon male motor performance. *Anim. Behav.* 79:771-778.
- Catchpole CK, Slater PJB. 2008. Bird song biological themes and variations. Cambridge University Press, Cambridge.

- Chesser RT, Banks RC, Barker FK, Cicero C, Dunn JL, Kratter AW, Lovette IJ, Rasmussen PC, Remsen Jr JV, Rising JD, Stotz DF, Winker K. 2009. Fifty-first supplement to the American Ornithologists' Union check-list of North American birds. *Auk* 127:726-744.
- Christie PJ, Mennill DJ, Ratcliffe LM. 2004. Pitch shifts and song structure indicate male quality in the dawn chorus of black-capped chickadees. *Behav. Ecol. Sociobiol.* 55:341-348.
- Collins S. 2004. Vocal fighting and flirting: the functions of birdsongs. In: *Nature's music, the science of bird song* (Marler P, Slabbekoorn H, eds). Elsevier Academic Press, New York. Pp. 39-79.
- Conner D A. 1982. Dialects versus geographic variation in mammalian vocalizations. *Anim. Behav.* 30:297-298.
- Cracraft J. 1989. Speciation and its ontology: the empirical consequence of alternative species concepts for understanding patterns and processes of differentiation. In: *Speciation and its consequences* (Otte D, Endler JA, eds). Sinauer Associates, New York. Pp. 28-59.
- Dabelsteen T. 2005. Public, private or anonymous? Facilitating and countering eavesdropping. In: *Animal communication networks* (McGregor PH, ed). Cambridge University Press, Cambridge. Pp. 38-62.
- DaCosta JM, Spellman GM, Escalante P, Klicka J. 2009. A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *J. Avian Biol.* 40:206-216.
- Dale J, Lank DB, Reeve HK. 2001. Signaling individual identity versus quality: a model and case studies with ruffs, queleas, and house finches. *Am. Nat.* 158:75-86.
- Dallmann R, Geissmann T. 2001. Different levels of variability in the female song of wild silvery gibbons (*Hylobates moloch*). *Behaviour* 138:629-648.
- Davidson SM, Wilkinson GS. 2002. Geographic and individual variation in vocalizations by male *Saccopteryx bilineata* (Chiroptera: Emballonuridae). *J. Mammal.* 83:526-535.
- De Queiroz K. 2005. Ernst Mayr and the modern concept of species. *Proc. Nat. Acad. Sci.* 102:6600-6607.
- Dingle C, Halfwerk W, Slabbekoorn H. 2008. Habitat-dependent song divergence at subspecies level in the grey-breasted wood-wren. *J. Evol. Biol.* 21:1079-1089.
- Ellis JM. 2008. Decay of apparent individual distinctiveness in the begging calls of adult female white-throated magpie-jays. *Condor* 110:648-657.
- Eriksen A, Slagsvold T, Lampe HM. 2011. Vocal plasticity—are Pied Flycatchers, *Ficedula hypoleuca*, open-ended learners? *Ethology* 117:188-198.
- Ey E, Fisher J. 2009. The “acoustic adaptation hypothesis” a review of the evidence from birds, anurans and mammals. *Bioacoustics* 19:21-48.
- Feng AS, Riedet T, Arch VS, Yu Z, Xu Z, Yu X, Shen J. 2009. Diversity of the vocal signals of concave-eared torrent frogs (*Odorrana tormota*): evidence for individual signatures. *Ethology* 115:1015-1028.
- Forrest TG. 1994. From sender to receiver: propagation and environmental effects on acoustic signals. *Am. Zool.* 34:644-654.
- Garnett ST, Christidis L. 2007. Implications of changing species definitions for conservation purposes. *Bird Conserv. Int.* 17:187-195.

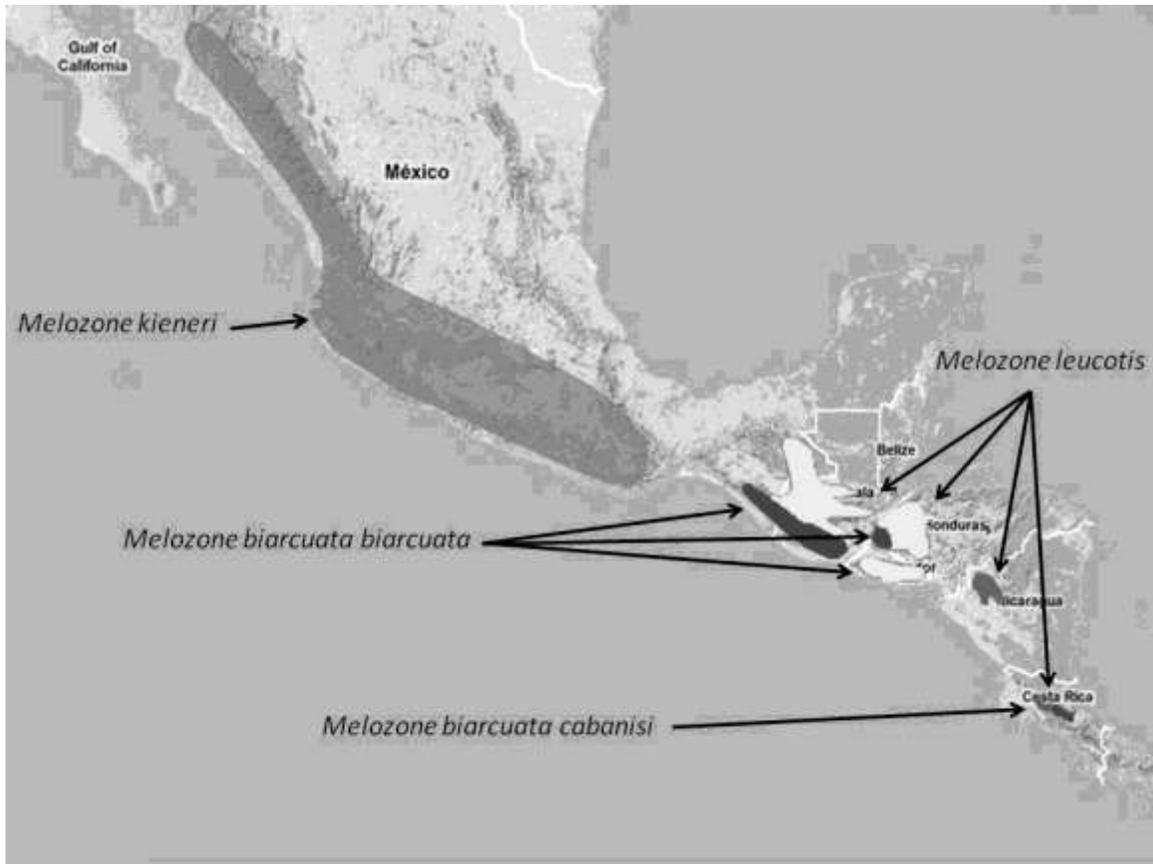
- Gasser H, Amézquita A, Hödl W. 2009. Who is calling? Intraspecific call variation in the arboreal frog *Allobates femoralis*. *Ethology* 115:596-607.
- Gerhardt H C. 1994. The evolution of vocalization in frogs and toads. *Annu. Rev. Ecol. Syst.* 25:293-324.
- Gil D, Gahr M. 2002. The honesty of bird song: multiple constraints for multiple traits. — *Trends Ecol. Evol.* 17:133-141.
- Grether GF. 2011. The neuroecology of competitor recognition. *Integr. Comp. Biol.* 51:807-818.
- Hansen P. 1979. Vocal learning: its role in adapting sound structures to long-distance propagation and a hypothesis on its evolution. *Anim. Behav.* 27:1270-1271.
- Harris MA, Lemon RE. 1972. Songs of song sparrows (*Melospiza melodia*): individual variation and dialects. *Canadian J. Zool.* 50:301-309.
- Howell SNG, Webb S. 1995. A guide to the birds of Mexico and northern Central America. Oxford University Press, New York.
- Isler ML, Cuervo AM, Bravo GA, Brumfield RT. 2012. An integrative approach to species-level systematics reveals the depth of diversification in an Andean thamnophilid, the Long-tailed Antbird. *Condor* 114:571-583.
- Jiggins CD, Mallarino R, Willmott KR, Bermingham E. 2006. The phylogenetic pattern of speciation and wing pattern change in Neotropical *Ithomia* butterflies (Lepidoptera: Nymphalidae). *Evolution* 60:1454-1466.
- Johnson RR, Haight LT. 1996. Canyon Towhee (*Melospiza fuscus*). In: The birds of North America Online (Poole A, ed) Cornell Lab of Ornithology, Ithaca.
- Krams I. 2001. Perch selection by singing chaffinches: a better view of surroundings and the risk of predation. *Behav. Ecol.* 12:295-300.
- Kroodsma D. 2004. The diversity and plasticity of birdsong. In: Nature's music: the science of bird song (Marler P, Slabbekoorn H, eds). Elsevier Academic Press, New York. Pp. 108-131.
- Lovell SF, Lein MR. 2004. Song variation in a population of Alder flycatchers. *J. Field Ornithol.* 75:146-151.
- Marler PA. 2004. Bird calls: a cornucopia from communication. In: Nature's music: the science of bird song (Marler P, Slabbekoorn H, eds). Elsevier Academic Press, New York. Pp. 132-177.
- Mathevon N, Dabelsteen T, Blumenrath SH. 2005. Are high perches in the blackcap *Sylvia atricapilla* song or listening posts? A sound transmission study. *J. Acoust. Soc. Am.* 117:442-449.
- Mayr E. 1969. Principles of systematic zoology. McGraw-Hill, New York.
- MacDougall-Shackleton EA, MacDougall-Shackleton SA. 2001. Cultural and genetic evolution in mountain white-crowned sparrows: song dialects are associated with population structure. *Evolution* 55:2568-2575.
- McGregor PK, Krebs JR. 1984. Sound degradation as distance cue in great tit (*Parus major*). *Behav. Ecol. Sociobiol.* 19:57-63.
- McGregor PK, Peake TM. 2000. Communication networks: social environments for receiving and signalling behaviour. *Acta Ethol.* 2:71-81.

- Morton ES. 1975. Ecological sources of selection on avian sounds. *Am. Nat.* 109:17-34.
- Nottebohm F, Nottebohm ME, Crane LA, Wingfield JC. 1987. Seasonal changes in gonadal hormone levels of adult male canaries and their relation to song. *Behav. Neural Biol.* 47:197-211.
- Nowicki S, Westneat M, Hoese W. 1992. Birdsong: motor function and the evolution of communication. *Sem. Neurosci.* 4:385-390.
- Ord JT, Stamps JA. 2009. Species identity cues in animal communication. *Am. Nat.* 174:585-593.
- Ord JT, King L, Young AR. 2011. Contrasting theory with the empirical data of species recognition. *Evolution* 65:2572-2591.
- Patten MA, Rotenberry JT, Zuk M. 2004. Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. *Evolution* 58:2144-2155.
- Peterson AT. 2006. Taxonomy is important in conservation: a preliminary reassessment of Philippine species-level bird taxonomy. *Bird Conserv. Int.* 16:155-173.
- Peterson AT. 2007. Philippine bird taxonomy and conservation: a response to Collar. *Bird Conserv. Int.* 17:115-118.
- Podos J. 2001. Correlate evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409:185-187.
- Ptacek MB. 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behav. Proc.* 51:111-134.
- Qvarnström A, Haavie J, Saether SA, Eriksson D, Pärt T. 2006. Song similarity predicts hybridization in flycatchers. *J. Evol. Biol.* 19:1202-1209.
- Redondo P, Barrantes G, Sandoval L. 2013. Urban noise influences vocalization structure in the house wren *Troglodytes aedon*. *Ibis* 155:621-625.
- Rising JD. 2011. Family Emberizidae (buntings and New World sparrows). In: *Handbook of the birds of the world, Volume 16 tanagers to New World blackbirds* (del Hoyo J, Elliot A, Christie D, eds). Lynx Edicions, Barcelona. Pp 428-683.
- Salinas-Melgoza A, Wright TF. 2012. Vocal learning and limited dispersal as dual mechanisms for dialect maintenance in a parrot. *PLOS One* 7:e48667.
- Sandoval L, Barrantes G. 2012. Characteristics of male spot-bellied bobwhite (*Colinus leucopogon*) song during territory establishment. *J. Ornithol.* 153:547-554.
- Sandoval L, Escalante I. 2011. Song description and individual variation in males of the Common Pauraque (*Nyctiodromus albicollis*). *Ornithol. Neotrop.* 22:173-185.
- Sandoval L, Mennill DJ. 2012. Breeding biology of white-eared ground-sparrow (*Melospiza leucotis*), with a description of a new nest type. *Ornithol. Neotrop.* 23:225-234.
- Sandoval L, Mennill DJ. 2013. Morphometric measurements permit accurate sexing of three species of Mesoamerican ground-sparrow (Genus: *Melospiza*). *Wilson J. Ornithol.* 125:471-478.
- Seddon N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution* 59:200-215.

- Seddon N, Tobias JA. 2010. Characteristics displacement from the receiver's perspective: species and mate recognition despite convergent signals in suboscine birds. *Proc. R. Soc. B* 277:2475-2483.
- Sewall KB. 2009. Limited adult vocal learning maintains call dialects but permits pair-distinctive calls in red crossbills. *Anim. Behav.* 77:1303-1311.
- Sewall KB. 2011. Early learning of discrete call variants in red crossbills: implications for reliable signaling. *Behav. Ecol. Sociobiol.* 65:157-166.
- Slabbekoorn H, Smith TB. 2002. Habitat-dependent song divergence in the Little Greenbul: an analysis of environmental selection pressures on acoustical signals. *Evolution* 56:1848-1858.
- Slabbekoorn H, Eilers J, Smith TB. 2002. Birdsong and sound transmission: the benefits of reverberations. *Condor* 104:564-573.
- Stiles FG, Skutch AF. 1989. *Guide to the birds of Costa Rica*. Cornell University Press, Ithaca.
- Tibbetts EA, Dale J. 2007. Individual recognition: it is good to be different. *Trends Ecol. Evol.* 22:529-537.
- Tobias JA, Seddon N. 2009. Signal design and perception in *Hypocnemis* antbirds: evidence for convergent evolution via social selection. *Evolution* 63: 3168-3189.
- Tobias JA, Seddon N, Spottiswoode CN, Pilgrim JD, Fishpool LDC, Collar NJ. 2010. Quantitative criteria for species delimitation. *Ibis* 152:724-746.
- Thomas JA, Stirling I. 1983. Geographic variation in the underwater vocalizations of Weddell seals (*Leptonychotes weddelli*) from Palmer Peninsula and McMurdo Sound, Antarctica. *Can. J. Zool.* 61:2203-2212.
- Tubaro PL, Segura ET. 1994. Dialect differences in the song of *Zonotrichia capensis* in the southern Pampas: a test of the acoustic adaptation hypothesis. *Condor* 96:1084-1088.
- Tweit RC, Finch DM. 1994. Abert's Towhee (*Melospiza aberti*). In: *The birds of North America Online* (Poole A, ed). Cornell Lab of Ornithology, Ithaca.
- Vannoni E, McElligott AG. 2007. Individual acoustic variation in fallow deer (*Dama dama*) common and harsh groans: a source-filter theory perspective. *Ethology* 113:223-234.
- Vargas-Castro, LE, Sánchez NV, Barrantes G. 2012. Repertoire size and syllable sharing in the song of the Clay-coloured Thrush (*Turdus grayi*). *Wilson J. Ornithol.* 124:446-453.
- West-Eberhard MJ. 1983. Sexual selection, social competition and speciation. *Q. Rev. Biol.* 58:155-183.
- Wiley EO. 1978. The evolutionary species concept reconsidered. *Syst. Zool.* 27:17-26.
- Wiley EO, Mayden, RL. 2000. The evolutionary species concept. In: *Species concepts and phylogenetic theory: a debate, 2000* (Wheeler QD, Meier R, eds). Columbia Press, New York. Pp. 70-89.
- Wiley RH. 1991. Association of song properties with habitats for territorial oscine birds of eastern North America. *Am. Nat.* 138:973-993.
- Wright TF, Wilkinson GS. 2001. Population genetic structure and vocal dialects in an amazon parrot. *Proc. R. Soc. Lond. B*: 268:609-616.

Wright TF, Dahlin CR, Salinas-Melgoza A. 2008. Stability and change in vocal dialects of the yellow-naped amazon. *Anim. Behav.* 76:1017-1027.

## Figures



**Figure 1.1.** Map showing the distribution of the three Mesoamerican *Melospiza* ground-sparrows that are the focus of this dissertation. Medium grey: Rusty-crowned Ground-sparrow, *Melospiza kieneri*. Pale grey: Prevost's Ground-sparrow, *Melospiza biarcuata*. Dark grey: White-eared Ground-sparrow, *Melospiza leucotis*. Source of distribution map data: Neotropical Birds (<http://neotropical.birds.cornell.edu/portal/species>).

## Chapter 2

### **Description of the vocalizations and vocal behaviour of White-eared Ground-sparrows (*Melospiza leucotis*) during the breeding season\***

---

\*This chapter is the outcome of joint research with C. Méndez and D. Mennill

### **Chapter summary**

There are few quantitative descriptive studies of the vocalizations and vocal behaviour of tropical bird species, in spite of the tropics' rich avian biodiversity and the extensive variety of vocalizations produced by tropical birds. This lack of information limits our understanding of tropical animals, including our ability to perform comparative analyses on vocal behaviours from an evolutionary perspective. In this study we present the first quantitative description of the vocal repertoire and daily vocal activity of White-eared Ground-sparrows (*Melospiza leucotis*), using focal and autonomous recordings collected during two consecutive breeding seasons in Costa Rica. We classified vocalizations into categories based on their visual appearance on sound spectrograms, creating a library of vocalizations for this species. We found that White-eared Ground-sparrows produce three main categories of vocalizations: solo songs, calls, and duets. Solo songs are produced only by males. Each male has a mean repertoire of  $3.5 (\pm 0.3)$  solo song types, which all share the same general structure with short introductory notes, a frequency-modulated middle section, and a terminal trill. Both sexes produce calls and coordinated vocal duets. We quantified patterns of diel variation in each category of vocalization, and found that ground-sparrows produce all three vocalizations at higher output at dawn (between 0500 and 0600) compared to the rest of the day. This study allowed us to conduct the first comparisons of vocalizations between White-eared Ground-sparrows and North American species in the genus *Melospiza*, and revealed both similarities and differences between the species groups. Our study also shows that vocalizations related to communication within pairs and territory defence (calls and duets) exhibited lower levels of individual distinctiveness than vocalizations related to female attraction (male solo songs). Our observations suggest that each of the three described

vocalizations have multiple functions in this species, revealing diverse communication functions with a small vocal repertoire in this tropical songbird.

## Introduction

A vocal repertoire is the complete library of vocalizations an individual or species can produce (Catchpole and Slater 2008). In the Order Passeriformes (songbirds), members of the Oscine suborder generally have larger vocal repertoires than members of the Suboscine suborder. Oscines learn their vocalizations culturally, whereas Suboscine appears to inherit their vocalizations genetically (Kroodsma 2004; Beecher and Brenowitz 2005). The result of the cultural learning process is that each population or individual produces new vocal characters by learning, making mistakes, or improvising (Beecher and Brenowitz 2005, Wright et al. 2008), thereby increasing the species' vocal repertoire through the time. Oscines in the tropics also have larger vocal repertoires than their counterparts from temperate habitats, because tropical birds exhibit several vocal behaviours that are rare or absent in the north temperate zone, including female solo songs (Langmore 1998; Beecher and Brenowitz 2005), pair duets (Hall 2004; 2009), and choruses (e.g., Baker 2004; 2009; Hale 2006). The number of quantitative descriptive studies that have been conducted on the vocalizations of tropical bird species is limited considering the diversity of tropical bird species. Moreover, with the recent importance assigned to vocalizations as a tool for resolving taxonomic issues between closely related taxa (Price and Lanyon 2002; Stiles 2009; Cadena and Cuervo 2010; Millsap et al. 2011), studies of this type are becoming increasingly recommended. In this study, we present the first quantitative description of vocal repertoire and daily vocal activity of White-eared Ground-sparrows (*Melospiza leucotis*). This is one of the seven recognized species in the genus *Melospiza* (Chesser et al. 2010), and one of four species in the genus where our knowledge of vocal behaviour is restricted to brief and anecdotal descriptions (Stiles and Skutch 1989; Howell and Webb 1995; Sandoval and Mennill 2012; see Chapter 6 for an exception). White-eared Ground-sparrows are

found between 500 and 2000 m a.s.l., from Chiapas-Mexico in the north to Costa Rica's Central Valley in the south (Stiles and Skutch 1989; Howell and Webb 1995). Across their distribution, this ground-sparrow inhabits thickets, secondary forest edges, and shade coffee plantations (Stiles and Skutch 1989; Howell and Webb 1995; Sandoval and Mennill 2012). White-eared Ground-sparrow pairs defend their territories year-round using mainly vocalizations (Sandoval and Mennill 2012; Chapter 5).

To expand our knowledge on the vocal repertoire and its function in White-eared Ground-sparrows, we had three objectives in this paper. (1) We sought to quantitatively describe the vocal repertoire of White-eared Ground-sparrows. (2) We sought to study the behavioural contexts in which the different types of vocalizations are used. (3) We sought to describe the pattern of diel variation in vocal output for each of these vocalizations.

## **Methods**

### Recording techniques

We collected recordings from birds in four populations of colour-banded White-eared Ground-sparrows in Costa Rica: Monteverde, Puntarenas Province (10°18'N, 84°48'W; altitude 1600 m), North Heredia, Heredia Province (10°01'N, 84°05'W; elevation: 1200-1500 m), University of Costa Rica campus, San Jose Province (09°56'N, 84°05'W; elevation 1200 m), and Lankester Botanical Garden, Cartago Province (09°50'N, 83°53'W; altitude 1400 m). We used two recording techniques. First, we directly recorded vocalizations during two consecutive breeding seasons (from April to August 2011 and from March to July 2012) by following and continuously recording focal birds during a 1 hour period, starting just before sunrise. These recordings were

collected using a shotgun microphone (Sennheiser K6/ME66) and a digital recorder (Marantz PMD 661 or PMD 660; recording format: WAVE; sampling rate: 44.1 kHz; 16 bit accuracy). We recorded 50 different pairs with this approach: 20 pairs were recorded in 2011 on two occasions ( $n = 14$ ) or three occasions ( $n = 6$ ), and 45 pairs were recorded in 2012 on one occasion ( $n = 43$ ) or two occasions ( $n = 2$ ). From the 45 pairs recorded in 2012, 15 were also recorded in 2011. Whenever possible, we noted the sex of the singer, although the thick vegetation and secretive nature of the birds made sex identification challenging. Therefore, some of the comparisons between vocal characteristics were made between pairs and not between individuals.

To quantify the diel pattern of vocalizations produced by pairs of White-eared Ground-sparrows, we also recorded birds using autonomous digital recorders (Wildlife Acoustics' Song Meters; Wildlife Acoustics Inc., Concord, MA, USA; recording format: WAVE; sampling rate: 44.1 kHz; accuracy: 16 bits). Each recorder was positioned in the centre of a pair's territory, to minimize the chance of recording neighbouring birds. Comparison of the autonomous recordings to the vocal repertoires collected during focal recordings confirmed that our autonomous recordings sampled the intended birds. We collected autonomous recordings from 0450 h (10 min before sunrise) until 1800 h. Based on preliminary recordings conducted across 24 h periods, we never detected vocalizations outside of this period. We recorded three pairs for five days, two pairs for three days, and nine pairs for two days during 2011 for the analysis of diel variation.

#### Vocalization measurements

Vocalizations were classified visually according to their appearance on sound spectrograms in Raven Pro 1.4 sound analysis software (Cornell Lab of Ornithology, Ithaca, NY, USA) following an

approach similar to that used by Franco and Slabbekoorn (2009). The vocal repertoire we recorded fit neatly into distinctions of calls, solo songs, and duets. We defined calls as short-duration vocalizations (duration  $\leq 1$  s) produced by both members of the pair (Figure 2.1); solo songs as the vocalizations produced solely by males (duration  $> 1$  s) and with 2 or more element types (Figure 2.2); and duets as vocalizations produced by both members of the pair (duration  $> 1$  s) involving the production of several elements that overlapped in time and frequency (Figure 2.3). Contrary to the majority of duetting species that have been studied in detail (Hall 2004), White-eared Ground-sparrows produce duets with vocalizations different from their solo songs (Figure 2.3). Occasionally, the vocalization that birds produce during duets was given by a lone individual and the partner did not respond; we refer to these vocalizations as “incomplete duets”, because our observations suggest that the main function for this vocalization is duet production.

We extracted vocalizations from our recordings and measured the following spectro-temporal details: duration (s), minimum frequency (Hz), maximum frequency (Hz), and frequency of maximum amplitude (Hz). We took these measurements using a combination of visualizations of the vocalizations, including the spectrogram, the power spectrum, and the waveform screens on Raven Pro 1.4, simultaneously. This approach offers the opportunity for obtaining more accurate values of frequency and duration than using the spectrogram alone, because power spectra and waveforms are not affected by the settings on the grey-scale (Charif et al. 2004). Spectrograms were constructed using a Hann window with 50 % overlap and 256 Hz transform size, resulting in a temporal resolution of 5.8 ms and a frequency resolution of 188 Hz. We collected these measurements only on vocalizations with high signal-to-noise ratio and without overlap by other sounds.

We annotated the total number of unique song types produced by each male, to

estimate each male's song repertoire. Song types were classified visually according to the number of different solo song elements and their appearance on spectrograms. Solo songs that showed variation only in the number of elements in the first part or final part of solo songs were classified as the same song type. We estimated song repertoire sizes using a curve-fitting method by applying the equation proposed by Wildenthal (1965). The estimation was conducted in Excel 2007 on individuals with  $\geq 80$  recorded songs recorded during the focal and autonomous recordings combined.

We analyzed diel variation in the vocal output by annotating the number of each vocalization type recorded from 0450 to 1800 h from the autonomous recorders. Birds typically produced their first vocalization of the day around 0500 h, although occasionally just prior to 0500 h, as early as 0450 h. We grouped the vocalizations from 0450 to 0500 h with the vocalizations produced from 0500 to 0600 h for this analysis. All other vocalizations were grouped into one-hour intervals from 0600 to 1800 h.

### Statistical analyses

We tested whether calls and duets showed distinctiveness between pairs using a discriminant function analysis (DFA). In this analysis we used the four spectro-temporal measurements as response variables, and pair identity as the independent variable. The accuracy of classification by pair was estimated using a Jackknife cross-validation method. We conducted this analysis separately for the two types of calls that we found in our recordings (see Results) and for complete duets. We used a binomial test to analyze the probability that the classification accuracy of the DFA is higher than the classification expected by chance (one divided by the number of pairs included in each DFA).

Since the number of elements inside each solo song type showed subtle variation, we conducted a Levene's test to compare the consistency in the number of elements in each male solo song type. For this analysis, we divided male solo songs into three components (see Results). Given that the number of elements in each solo song component varied according to the song type, using the raw data in this analysis would artificially increase the variation between song types. To control for this variation, we first estimated the average variance of each song component per song type; we then used the average value per song component as our response variable in the test, and song type as the independent variable.

We assessed whether males delivered song types in a predictable or random order using a Markov chain analysis as in Lemon and Chatfield (1971). This method allowed us to analyze the probability that each song type was sung in a random order (no preferred transition between songs types; Leonardo and Konishi 1999). This test reports a single value for each male; however, since we were interested in patterns across the population, we conducted two extra tests. First, we conducted multiple regression analysis to demonstrate that the results of our Markov chain analysis were not influenced by differences in the total number of songs recorded and the repertoire size of each male. In this analysis, we included the number of song types recorded and the total number of recorded songs as independent variables, and the probability of singing in a random order (as calculated for each male with the Markov chain approach) as the response variable. Given that some males produce their song types in a predictable order and others in random order (see Results), we conducted a binomial test to evaluate which type of singing behaviour occurred more often in the population.

To analyze diel variation in vocal behaviour, we count the total number of vocalizations per hour per individual from 0500 to 1800 hours. Then we tested for the total occurrence of each vocalization category throughout the day using linear mixed-models. In this analysis, the

fixed factor was the hour of the day. The response variables were the total number of each vocalization category produced per hour per individual. Finally, we used subject identity as a random factor to control for multiple values for the same subject.

All descriptive statistics are reported as mean  $\pm$  SE. We conducted linear mixed-models in JMP (version 7.0 SAS Institute, Cary, NC, U.S.A.), the Markov chain test in PAST (version 2.14; Øyvind Hammer, Natural History Museum, University of Oslo, Norway), and the rest of the tests in Systat (version 11.00.01; SYSTAT Software, Chicago, IL, U.S.A.).

## Results

### Calls

White-eared Ground-sparrows produced two types of call. The first type of call, which we refer to hereafter as the “*chip*” call, is a single, short-duration, high-frequency, broadband note (Figure 2.1). The second type of call, which we refer to hereafter as a “*tseet*” call, is a single narrow-bandwidth note that gradually ascends or descends (Figure 2.1). Both types of calls were produced by males and females, and varied in the frequency of production ( $114.10 \pm 15.03$  *chip* calls per hour per pair;  $8.72 \pm 1.44$  *tseet* calls per hour per pair based on  $1.84 \pm 0.15$  h focal recordings of 49 pairs).

On average, the duration of the *chip* call was  $0.08 \pm 0.003$  s (range = 0.06 – 0.09 s, CV = 25.4 %), with a minimum frequency of  $7326 \pm 62$  Hz (range = 6833 – 8184 Hz, CV = 5.9 %), a maximum frequency of  $12,345 \pm 109$  Hz (range = 10,189 – 13,314 Hz, CV = 6.1 %), and a frequency of maximum amplitude of  $8315 \pm 77$  Hz (range = 7475 – 8892 Hz, CV = 6.5 %). We observed the ground-sparrows using this vocalization in two main contexts: as a contact call

when foraging, and when they were excited after a potential predator was close to the nest or chicks.

On average, the duration of the *tseet* call was  $0.28 \pm 0.013$  s (range = 0.16 – 0.40 s, CV = 27.6 %), with a minimum frequency of  $8356 \pm 107$  Hz (range = 6833 – 9111 Hz, CV = 7.9 %), a maximum frequency of  $10,520 \pm 109$  Hz (range = 9273 – 11,537 Hz, CV = 6.4 %), and a frequency of maximum amplitude of  $9305 \pm 91$  Hz (range = 7755 – 10,565 Hz, CV = 6.0 %). We observed the ground-sparrows using this vocalization in two main contexts: before starting a duet, and when both individuals were far away from each other.

Both call types showed a low level of individual distinctiveness based on the pair that produced the vocalization. In a discriminant analysis with cross-validation, *chip* calls (Wilks'  $\lambda = 0.35$ ,  $F_{192,36697} = 57.0$ ,  $p < 0.001$ ) were correctly classified to the pair that produced the call 18 % of the time, significantly higher than the 2.08 % expected by chance (binomial test:  $p < 0.001$ ; in comparison to chance expectation of 1 divided by 48 pairs included in the analysis). Similarly, *tseet* calls (Wilks'  $\lambda = 0.24$ ,  $F_{148,2611} = 7.5$ ,  $p < 0.001$ ) were correctly classified to the pair that produced the call 32 % of the time, significantly higher than the 2.63 % expected by chance (binomial test:  $p < 0.001$ ; in comparison to chance expectation of 1 divided by 38 pairs included in the analysis).

### Solo Songs

Over two years of field study we never detected a female producing this vocalization. Male solo songs were variable and readily classifiable into distinct song types (Figure 2.2). We detected 33 unique song types in our sample of 3133 analyzed songs (Figure 2.2). Most male solo songs had three sections: (1) songs began with high frequency elements similar to *chip* calls; (2) the middle

section of songs contained frequency-modulated elements; and (3) songs concluded with a short trill. Of the 33 song types we detected, there were two song types that lacked a frequency-modulated middle section (Figure 2.2). Among song types, we found substantial variation in the number of song elements in the terminal trill section ( $\sigma^2 = 11.90$ ), with less variation in the number of elements in the middle section ( $\sigma^2 = 0.26$ ), and finally the least variable number of elements in the introductory section ( $\sigma^2 = 0.01$ ; Levene's test:  $F = 6.5$ ,  $p = 0.003$ ). Solo songs showed an average duration of  $1.9 \pm 0.1$  s (range = 0.6 – 3.1 s, CV = 16.4 %), a minimum frequency of  $3535 \pm 124$  Hz (range = 1595 – 5769 Hz, coefficient of variation: CV = 20.2 %), a maximum frequency of  $11,209 \pm 161$  Hz (range = 6220 – 13,801 Hz, CV = 8.3 %), and frequency of maximum amplitude of  $5956 \pm 106$  Hz (range = 2498 – 9216 Hz, CV = 10.2 %).

The solo song repertoire size of White-eared Ground-sparrows, calculated on the basis of 19 males where we recorded 80 or more songs, varied from two to eight songs ( $3.5 \pm 0.3$  songs; Figure 2.4). A significant majority of White-eared Ground sparrow males delivered their song with immediate variety (binomial test:  $p = 0.02$ ), with 24 males delivering their songs in a random order and 14 males in a predictable order (test of random order based on Markov chain approach). These results were not influenced by the repertoire size or the total number of songs recorded for each male (multiple regression:  $F_{2,35} = 3.0$ ,  $p = 0.064$ ,  $r^2 = 0.15$ ).

### Duets

Pairs of White-eared Ground-sparrows produced a third type of vocalization that was given chiefly as coordinated vocal duets (Figure 2.3). Unlike some other tropical birds (e.g. Mann et al. 2003; Mennill and Vehrencamp 2005; Logue 2006), the vocalizations that birds contributed to duets were wholly different from the vocalizations used by males as solo songs in their spectro-

temporal features (Figures 2.2 and 2.3). Based on our observations of colour-banded pairs, both sexes created duets (i.e. responded to their partner's duet contribution, turning it from a solo into a duet). From a total of 812 recordings of this vocalization obtained during the focal recording sessions, we found that this vocalization was produced as a duet 71.68 % of the time, and the remaining as a vocalization by just one individual of the pair, i.e. an "incomplete duet" (Figure 2.3).

Duets started with a series of one to six introductory elements ( $2.31 \pm 0.13$  elements) with longer inter-element intervals than the main duet elements (Figure 2.3). Introductory duet elements varied from arc shaped to a slight upward slope on the spectrogram (Figure 2.3). The main elements in duets were the elements that overlapped between both individuals of the pair. They were broadband and noisy elements (Figure 2.3). The vocalizations of the two individuals overlapped for  $3.64 \pm 0.22$  s, on average. Duets were produced by the second individual overlapping the first one in both frequency and time (i.e. polyphonal duets; Figure 2.3). The second bird concluded its song an average  $1.57 \pm 0.17$  s after the first bird. Complete duets ( $5.77 \pm 0.20$  s, range = 3.79 – 8.42 s, CV = 24.0 %) were longer than incomplete duets ( $4.33 \pm 0.24$  s, range = 1.85 – 8.75 s, CV = 33.4 %;  $t = 6.44$ ,  $df = 37$ ,  $p < 0.001$ ). Complete duets ( $5093 \pm 14$  Hz, range = 3940 – 6046 Hz, CV = 14.4 %) showed higher minimum frequencies than incomplete duets ( $5205 \pm 142$  Hz, range = 3792 – 6409 s, CV = 16.8 %;  $t = -3.77$ ,  $df = 37$ ,  $p = 0.001$ ). Complete and incomplete duets showed similar values of maximum frequency ( $t = 0.29$ ,  $df = 37$ ,  $p = 0.78$ ; complete:  $11,547 \pm 80$  Hz, range = 10,405 – 12,605 Hz, CV = 4.8 %; incomplete:  $11,385 \pm 100$  Hz, range = 10,608 – 12,615 s, CV = 5.4 %), and frequency of maximum amplitude ( $t = 0.25$ ,  $df = 37$ ,  $p = 0.81$ ; complete:  $7444 \pm 169$  Hz, range = 6202 – 8829 Hz, CV = 15.7 %; incomplete:  $7609 \pm 188$  Hz, range = 4565 – 8807 s, CV = 15.2 %). Complete duets showed a low level of individual distinctiveness, with just 11 % of correct classification of duets based on the pair that produced

the vocalization (Wilks'  $\lambda = 0.26$ ,  $F_{188,2121} = 4.5$ ,  $p < 0.001$ ), significantly higher than the 2.08 % expected by chance (binomial test:  $p = 0.01$ ; in comparison to chance expectation of 1 divided by 48 pairs included in the analysis). No analysis of individual distinctiveness was conducted on incomplete duets, because for most incomplete duets we were unable to determine which individual of the pair had produced the sound.

#### Diel variation

White-eared Ground-sparrow vocal output varied through the day, with the highest output between 0500 and 0600 hours (LMM:  $F_{12,156} = 10.1$ ,  $p < 0.001$ ; Figure 2.5). We observed the same pattern of vocal output for calls ( $F_{12,156} = 3.5$ ,  $p < 0.001$ ; Figure 2.5), solo songs ( $F_{12,144} = 10.2$ ,  $p < 0.001$ ; Fig. 2.5), and duets ( $F_{12,156} = 32.4$ ,  $p < 0.001$ ; Figure 2.5). In the case of solo songs, we observed that males produced an overwhelming majority of their daily vocal output at the start of the day; males sang between the 82% and 93% of all songs in the first hour of the day (Figure 2.5). This effect was less pronounced in the other two types of vocalization: only 18 % to 31 % of calls and 33 % to 39 % of duets were produced during the first hour of the day (Figure 2.5).

White-eared Ground-sparrows vocalized during all daylight hours, with lowest output around noon (Figure 2.5). Songs were the first vocalizations produced in the day, with an average start time of 0505 hours  $\pm$  2.3 min, follow by calls at 0508 hours  $\pm$  0.9 min, and finally by duets at 0514 hours  $\pm$  3.4 min.

## Discussion

White-eared Ground-sparrows produce three main categories of vocalizations: two types of call, male solo songs, and duets. Our analyses, based on two years of focal and autonomous recordings from four populations in Costa Rica, substantiates previous anecdotal descriptions of vocalizations in this species, which report the occurrence of three main categories of vocalizations (Stiles and Skutch 1989; Howell and Webb 1995; Chapter 6). Our analyses allowed us to observe similarities and differences between vocalizations and between pairs. Calls and duets were very similar in acoustic structure between pairs, whereas solo songs were highly variable in spite of their consistent structure (introductory section, frequency-modulated middle section, and terminal trill section). We quantified the pattern of diel variation of each vocalization type during the breeding season, and found that all three vocalization types were produced at higher levels at the start of the day (0450 to 0600 hours) compared to the rest of the day.

Our bioacoustic analyses reveal, for the first time, that White-eared Ground-sparrows produce two distinct types of calls. Both call types were highly different in acoustic and temporal structure but were used in similar contexts. The *chip* call and *tseet* call were used as a pair contact signal when they foraged or moved separately inside the territory. We also observed that *chip* was used as an alarm signal when a potential predator was close (observed predators included pygmy owls, dogs, and snakes; birds behaved similarly when humans were close to the nest), or as a response to alarm calls by the bird's breeding partner. In the predation context, *chip* calls were produced at a higher rate than in a contact context. These two main contexts of White-eared Ground-sparrow calls have been reported as general functions of calls in several bird species (reviewed by Marler 2004). The dual function of *chip* call also occurs in other

species, including Chaffinches (*Fringilla coelebs*; Marler 1956) and Steller's Jays (*Cyanocitta stelleri*; Hope 1980), in which they vary the production rate or the duty cycle. The function of the differences in call rate in this ground-sparrow is still unknown, and therefore, future research is needed.

The acoustic structure of the *chip* call, with short duration and broad bandwidth, should make this vocalization easy to localize (Marler 2004), which supports the idea that this call serves as a contact or alarm call function. In the case of a contact signal function, the production of this call type will allow both individuals of the pair to know the location of the other within the dense vegetation of the territories of these ground-sparrows (Hale 2006; Sandoval and Barrantes 2012). In the case of an alarm signal, considering that the main predators inside thickets are stationary predators (e.g., small mammals, lizards, snakes, and avian ambush predators); this type of vocalization will be advantageous because it may communicate the position of the threat, possibly facilitating a mobbing response from conspecific and heterospecific individuals (Marler 2004). The acoustic structure of *tseet* may transmit better inside dense vegetation of thicket habitats due to the longer call duration and the narrower bandwidth than the *chip* call, characteristics that are known to favour sound transmission in dense vegetation (Wiley 1991; Bradbury and Vehrencamp 2011). Therefore, White-eared Ground-sparrows may benefit from using *tseet* call to communicate at larger distances between pair individuals than using *chip* call. Due to the acoustic characteristics of *tseet* call, it may be more difficult to localize the position of the signaler.

Solo songs were the most variable vocalization we recorded from White-eared ground-sparrows, with spectrotemporal details that varied between songs from the same male. Songs were easily classified into distinct types, which males produced with eventual variety. A total of

94 % (31 of 33 song types) of all recorded songs showed the same structure of three types of elements (introductory notes, frequency-modulated middle part, and terminal trill). The song structure in White-eared Ground-sparrows was very different than that reported for the congeneric Abert's (*M. aberti*), California (*M. crissalis*), and Canyon Towhee (*M. fuscus*), which all have solo songs composed of a single element repeated several times (Tweit and Finch 1994; Johnson and Haight 1996; Benedict et al. 2011). Yet the song structure we report here is quite similar to Prevost's Ground-sparrows, which are closely related to White-eared Ground-sparrows (DaCosta et al. 2009), in which there are several different elements in the songs (Chapter 6). These differences may reflect genetic divergence, since both groups of species are part of different clades inside the genus (DaCosta et al. 2009). Given the similarity between these two Mesoamerican Ground-sparrows, and their differences from northern Ground-sparrows, it is possible that song styles vary between the two major clades in this group. Alternatively, the acoustic differences may reflect adaptation to different habitats, because the northern towhee species within *Melospiza* occupy more open habitats where fast repetition of a single element may provide an acoustic advantage (Handford and Loughheed 1991; Wiley 1991; Naguib 2003).

Based on the number of solo song types sung by each White-eared Ground-sparrow male ( $3.5 \pm 0.3$  song types), this species has a small repertoire, according to the classification proposed by Garamszegi et al. (2005). However, one individual Canyon Towhee showed a repertoire of five song types (Marler and Isaac 1960), and ten individual Rusty-crowned Ground-sparrows (*M. kieneri*) showed repertoires that varied from four to ten song types (Chapter 3), indicating that small solo song repertoires may be the rule for the genus *Melospiza*.

We found that White-eared Ground-sparrows have a unique vocalization, completely distinct in acoustic structure from male solos songs, used chiefly for duetting. This pattern also occurs in other *Melospiza* species (Tweitt and Finch 1994; Johnson and Haight 1996; Benedict et al. 2011; Chapter 6). The acoustic structure of duets bears a similarity to the duets of other species in the genus, especially with the duets of Prevost's Ground-sparrows (Chapter 6), and to a lesser degree, with the duets of California Towhees (Benedict and McEntee 2009; Benedict 2010). As in these congeners, White-eared Ground-sparrow duets start with introductory elements with a narrower bandwidth and are followed by noisier elements with broad bandwidths. In the case of California Towhees, duets have a single and clear frequency-modulated element between the introductory and noisy elements that is not found in White-eared and Prevost's Ground-sparrow duets (Benedict and McEntee 2009; Chapter 6).

White-eared Ground-sparrow duets were created by both sexes responding to their partner's duet contribution. The frequency and time overlap within duets is similar to the duets of California Towhees (Benedict and McEntee 2009) and Rusty-crowned Ground-sparrows (Chapter 3), the only other species with a detailed description of this vocal behaviour. We observed duets being produced in two contexts. The first observed context was when a bird approached its breeding partner. This behaviour is consistent with the Maintaining Contact Hypothesis that states that pair individuals create a duet to indicate their location, and the Signalling Commitment Hypothesis that states that pair individuals create a duet to indicate the commitment between them (Hall 2004). This pattern has been quantified in two other duetting species, the Rufous-and-white Wren (*Thryophilus rufalbus*; Mennill and Vehrencamp 2008) and the Black-bellied Wren (*Pheugopedius fasciatoventris*; Logue 2007). The second context was when pairs responded to the duets of neighbouring pairs. This behaviour is consistent with the Joint Territorial Defence Hypothesis, which states that territorial pairs respond aggressively to

vocalizations of neighbours or individual conspecific strangers (Hall 2004, 2009). Playback studies in White-eared Ground-sparrows reinforce this function for duets, because it was the most common vocalization produced by territorial pairs as a response to simulated territory intrusions (Chapter 6).

Our analyses showed that calls and duets were very similar between pairs of White-eared ground-sparrows. This result may indicate that these vocalizations do not serve to communicate pair identity (or individual identity). However, in the case of calls it is possible that our methods inflated the variation, reducing the probabilities of finding differences between pairs, since we grouped the calls produced by both sexes of the pair as the same unit of comparison. A more detailed study that distinguishes the individuals that produce each call is necessary to discard the possibility that sex-differences in calls or duet contributions might obscure individually-distinctive differences in these vocalizations. Our results on low levels of individual distinctiveness in calls and duet contributions stand in contrast with the reported results for male solo songs in this ground-sparrow, which show that males have high individual distinctiveness based on both acoustic and structural characteristics (Chapter 5).

White-eared Ground-sparrows show a dramatic dawn chorus performance, where the overwhelming majority of vocalizations occur at the start of the day (i.e. 0450 to 0600 hours). High output of solo songs and duets early in the morning may contribute to territory defence against possible territorial intrusions, as has been suggested previously for other bird species (Staicer et al. 1996; Amrhein and Erne 2006; Koloff and Mennill 2013). The three types of vocalization showed differences in their pattern of diel variation, and this is probably related to the functions of each vocalization. Calls and duets were produced at similar levels after the first hour, which reinforces the proposed function for both vocalizations. Behaviours associated with

pair contact during foraging, displacement inside the territory, and individual approaches are not expected to have a peak during the day; therefore, we expected a parallel output for calls and duets throughout the day.

Males produced more than 88 % of the song output at the start of the day, before males and females started to conduct their usual pair behaviours (e.g., foraging and joint territory defence). Based on preliminary observations we have collected outside the breeding season, males apparently only sing during the breeding season, and song is routinely produced across all breeding stages (Chapter 5). This singing behaviour in White-eared Ground-sparrows is similar to the behaviour displayed by seasonal breeding bird species in the temperate forest, where the main function of the song is mate attraction during the reproductive season (Collins 2004; Catchpole and Slater 2008). Males of this ground-sparrow species, however, live in pairs year round (Sandoval and Mennill 2012), and may mate with the same female during several consecutive years (pers. obs.), reducing the probability that male solo song is used to attract a pair each year. Our observations suggest that males may sing to attract neighbouring females for extra-pair copulations, as occurs in other bird species that continue producing a dawn chorus performance after pair formation (Gibbs et al. 1990; Richardson and Burke 2001; Mennill et al. 2004). A detailed study that evaluates the occurrence of extra pair copulations is necessary to evaluate this assumption about the solo song output function in this species.

Quantitative descriptions of the vocalizations and vocal behaviours of birds are necessary to perform comparative studies to understand the evolution of vocalization types in closely related species. For example, this study on White-eared Ground-sparrows allowed us to conduct the first comparisons in term of vocalizations with the species in the genus *Melospiza*, and revealed similarities and differences between this species and its congeners. For calls, we

found that White-eared Ground-sparrows produced two call types that are similar in acoustic structure and context of use with the calls produced by Rusty-crowned Ground-sparrows (Chapter 3), Abert's Towhees (Tweit and Finch 1994), California Towhees (Benedict et al. 2011), and Canyon Towhees (Johnson and Haight 1996). The calls of the towhees appear to occur at lower frequencies (e.g., 1 to 4 kHz approximately; Tweit and Finch 1994, Johnson and Haight 1996, Benedict et al. 2011), than the ground-sparrows calls (this chapter; Chapter 3). For solo songs, we found that White-eared Ground-sparrows produced solo songs with three conspicuous parts (introductory high frequency elements similar to *chip* calls; the middle frequency-modulated elements; and a final trill), that differ from its congeners. The structure of Rusty-crowned Ground-sparrow solo songs is a mix of different trill elements with some frequency-modulate elements (Chapter 3), whereas the congeneric towhee species produce solo songs comprising trills (Tweit and Finch 1994, Johnson and Haight 1996, Benedict et al. 2011). The minimum frequency at which the solo song is produced for the ground-sparrows is higher than the frequency used by towhees, and the frequency bandwidth for ground-sparrows also is broader than in towhees. Solo songs appear to be used for female mate attraction in all of these *Melospiza* species, as well as territory defence in the towhees, which is a possible function in ground-sparrows that still needs to be tested. For duets, we found that White-eared Ground-sparrows, as in the other congeneric ground-sparrows and towhees, produced duets with vocalizations different from vocalizations used as solo songs. In all of the species that have been studied to date, duets appear to be used for pair contact and territory defence (Tweit and Finch 1994, Johnson and Haight 1996, Benedict et al. 2011, Chapter 3 and 7). The acoustic structure of White-eared Ground-sparrow duets is more similar to Prevost's Ground-sparrow than to Rusty-crown Ground sparrow and the congeneric towhee duets, and showed the longest duration of all of the recorded duets (Tweit and Finch 1994, Johnson and Haight 1996, Benedict et al. 2011,

Chapter 3 and 6). With respect to the pattern of diel variation, we found that White-eared and Rusty-crowned Ground-sparrows showed a parallelism in vocal output during the day with a single peak in the first hour of the day and substantially reduced vocal activity the rest of the day (Chapter 3). However, the White-eared Ground-sparrow produced more vocalizations per hour per individual during all hours of the day in comparison to Rusty-crowned Ground-sparrows (Chapter 3).

All described vocalizations showed multiple functions in this species, revealing that even with a small vocal repertoire it is possible to achieve several communication functions. Our study also showed that vocalizations that appear to be related to within-pair communication and territory defence (calls and duets) were more similar between pairs (i.e. had lower individual distinctiveness) than those reported between males singing solo songs (a vocalization more related with female attraction). This study expands our knowledge about the vocal diversity in Neotropical sparrows species, and will facilitate future experimental analyses to understand in more detail the function of each category of vocalization described here, as well as the importance of individual and population variation.

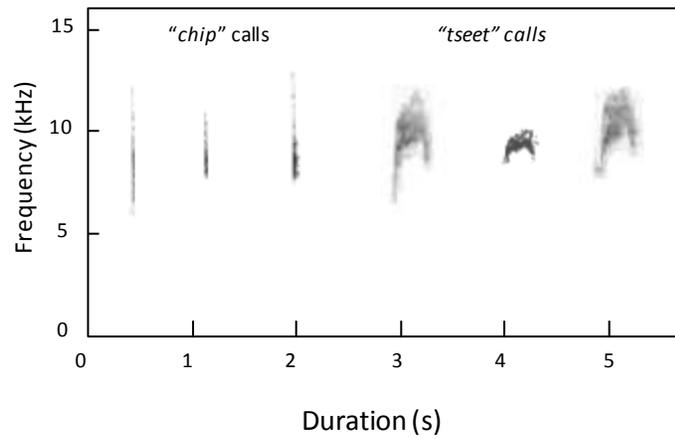
## References

- Amrhein V, Erne N (2006) Dawn singing reflects past territorial challenges in the Winter Wren. *Anim Behav* 71:1075–1080
- Baker MC (2004) The chorus song of cooperatively breeding laughing Kookaburras (*Coraciiformes, Halcyonidae: Dacelo novaeguineae*): characterization and comparison among groups. *Ethology* 110:21–35
- Baker MC (2009) Information content in chorus songs of the group-living Australian Magpie (*Cracticus tibicen dorsalis*) in Western Australia. *Ethology* 115:227–238
- Beecher MD, Brenowitz EA (2005) Functional aspects of song learning in songbirds. *Trends Ecol Evol* 20:143–149
- Benedict L (2010) California Towhee vocal duets are multi-functional signals for multiple receivers. *Behaviour* 147:953–978
- Benedict L, McEntee JP (2009) Context, structural variability and distinctiveness of California Towhee (*Pipilo crissalis*) vocal duets. *Ethology* 115:77–86
- Benedict L, Kunzmann MR, Ellison K, Purcell KL, Johnson RR, Haight LT (2011) California Towhee (*Melospiza crissalis*), In: Poole A (ed) *The birds of north America online*. Cornell Lab of Ornithology, Ithaca. Accessed 20 October 2013
- Bradbury JW, Vehrencamp SL (2011) *Principles of animal communication*. 2nd ed. Sinauer, Sunderland
- Cadena CD, Cuervo AM (2010) Molecules, ecology, morphology, and songs in concert: how many species is *Arremon torquatus* (Aves: Emberizidae)? *Biol J Linn Soc* 99:152–176
- Catchpole CK, Slater PJR (2008) *Bird song: biological themes and variations*. Cambridge University Press, Cambridge
- Charif R, Clark C, Frisrup K (2004) *Raven 1.2 User's Manual*. Cornell Laboratory of Ornithology, Ithaca
- Chesser RT, Banks RC, Barker FK, Cicero C, Dunn JL, Kratter AW, Lovette IJ, Rasmussen PC, Remsen JV Jr, Rising JD, Stotz DF, Winker K (2010) Fifty-First supplement to the American Ornithologists' Union check-list of North American birds. *Auk* 127:726–744
- Collins S (2004) Vocal fighting and flirting: the functions of birdsongs. In: Marler P, Slabbekoorn H (eds) *Nature's music, the science of bird song*. Elsevier Academic Press, San Diego, pp 39–79
- DaCosta JM, Spellman GM, Escalante P, Klicka J (2009) A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *J Avian Biol* 40: 206–216
- Franco P, Slabbekoorn H (2009) Repertoire size and composition in great tits: a flexibility test using playbacks. *Anim Behav* 77:261–269
- Garamszegi LZ, Balsby TJ, Bell BD, Borowiec M, Byers BE, Draganoiu T, Eens M, Forstemeier W, Galeotti P, Gil D, Gorissen L, Hansen P, Lampe HM, Leitner S, Lontkowski J, Nagle L, Nemeth E, Pinxten R, Rossi JM, Saino N, Tanvez A, Titus R, Török J, Van Duyse E, Møller AP (2005) Estimating the complexity of bird song by using capture-recapture approaches from community ecology. *Behav Ecol Sociobiol* 57:305–317

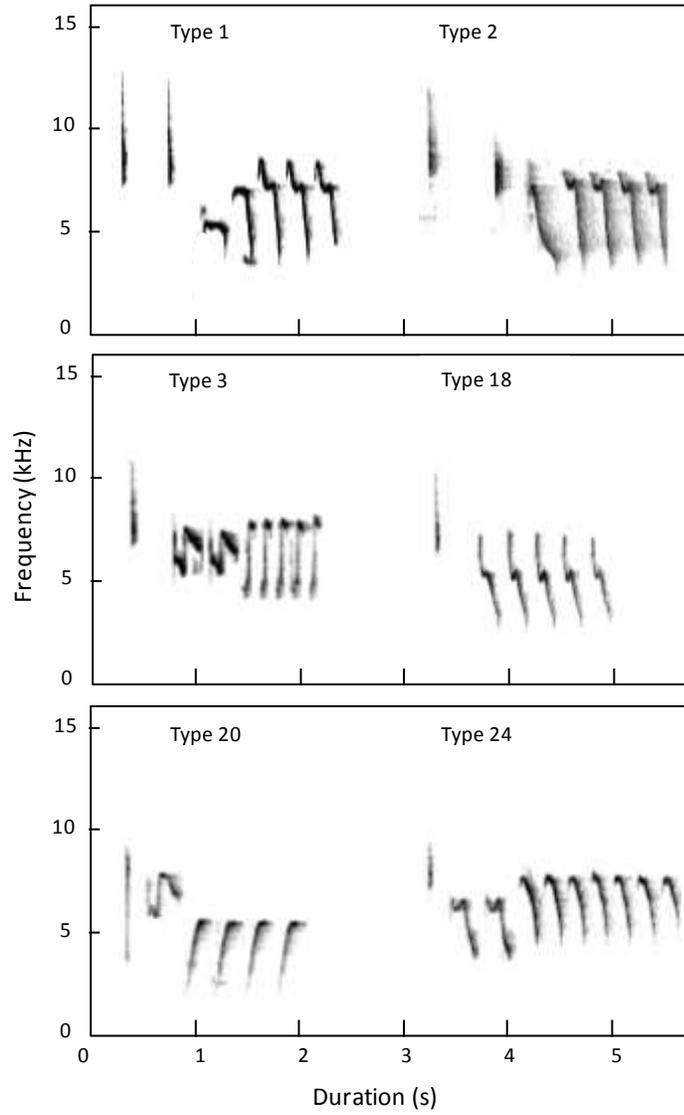
- Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ (1990) Realized reproductive success of polygynous Red-winged Blackbirds revealed by DNA markers. *Science* 250: 1394–1397
- Hale A (2006) Group living in the black-breasted wood-quail and the use of playbacks as a survey technique. *Condor* 108:107–119
- Hall ML (2004) A review of the hypotheses for the functions of avian duetting. *Behav Ecol Sociobiol* 55: 415–430
- Hall ML (2009) A review of vocal duetting in birds. *Ad Stud Behav* 40:67–121
- Handford P, Loughheed SC (1991) Variation in duration and frequency characters in the song of the Rufous-collared Sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects and body size. *Condor* 93: 644–658
- Hope S (1980) Call form in relation to function in the Steller's jay. *Am Nat* 116:788–820
- Howell SNG, Webb S (1995) A guide to the birds of Mexico and northern Central America. Oxford University Press, New York.
- Johnson RR, Haight LT (1996) Canyon Towhee (*Melospiza fuscus*). In: Poole A (ed) The birds of north America online. Cornell Lab of Ornithology, Ithaca. Accessed 20 October 2013
- Koloff J, Mennill DJ (2013) Vocal behaviour of Barred Antshrikes, a Neotropical duetting suboscine bird. *J Ornithol* 154:51–61
- Kroodsma D (2004) The diversity and plasticity of birdsong. In: Marler P, Slabbekoorn H (eds) Nature's music, the science of bird song. Elsevier Academic Press, San Diego, pp 108–131
- Langmore NE (1998) Functions of duet and solo songs of female birds. *Trends Ecol Evol* 13:136–140
- Lemon RE, Chatfield C (1971) Organization of song in cardinals. *Anim Behav* 19:1–17
- Leonardo A, Konishi M (1999) Decrystallization of adult birdsong by perturbation of auditory feedback. *Nature* 399: 466–470
- Logue DM (2006) The duet code of the female black-bellied wren. *Condor* 108: 326–335
- Logue DM (2007) Duetting in space: a radio-telemetry study of the black-bellied wren. *Proc R Soc B*: 274:3005–3010
- Mann NI, Marshall-Ball L, Slater PJB. 2003. The complex song duet of the Plain Wren. *Condor* 105: 672–682
- Marler P (1956) The voice of the chaffinch and its function as a language. *Ibis* 98:231–261
- Marler P (2004) Bird calls: a cornucopia from communication. In: Marler P, Slabbekoorn H (eds) Nature's music, the science of bird song. Elsevier Academic Press, San Diego, pp 132–177
- Mennill DJ, Vehrencamp SL (2005) Sex differences in the singing and duetting behavior of neotropical Rufous-and-white Wrens (*Thryothorus rufalbus*). *Auk* 122: 175–186
- Mennill DJ, Vehrencamp SL (2008) Context-dependent functions of avian duets revealed through microphone array recordings and multi-speaker playback. *Curr Biol* 18:1314–1319

- Mennill DJ, Ramsay SM, Boag PT, Ratcliffe LM (2004) Patterns of extrapair mating in relation to male dominance status and female nest placement in Black-capped Chickadees. *Behav Ecol* 15: 757–765
- Millsap BA, Seipke SH, Clark WS (2011) The Gray Hawk (*Buteo nitidus*) is two species. *Condor* 113:326–339
- Naguib M (2003) Reverberation of rapid and slow trills: implications for signal adaptations to long-range communication. *J Acoust Soc Am* 113:1749–1756
- Price JJ, Lanyon SM (2002) Reconstructing the evolution of complex bird song in the oropendolas. *Evolution* 56:1514–1529
- Richardson DS, Burke T (2001) Extra-pair paternity and variance in reproductive success related to breeding density in Bullock's Orioles. *Anim Behav* 62: 519–525
- Sandoval L, Barrantes G (2012) Characteristics of male spot-bellied bobwhite (*Colinus leucopogon*) song during territory establishment. *J Ornithol* 153:547–554
- Sandoval L, Mennill DJ (2012) Breeding biology of White-eared Ground-sparrow (*Melospiza leucotis*), with a description of a new nest type. *Ornithol Monographs* 23:225–234
- Staicer CA, Spector DA, Horn AG (1996) The dawn chorus and other diel patterns in acoustic signaling. In: Kroodsma DE, Miller EH (eds) *Ecology and evolution of acoustic communication in birds*. Cornell University Press, Ithaca, pp 426–453
- Stiles FG (2009). A review of the genus *Momotus* (Coraciiformes: Momotidae) in northern South America and adjacent areas. *Ornithol Colombiana* 8:29–75
- Stiles FG, Skutch AF (1989) *A guide to the birds of Costa Rica*. Cornell University Press, Ithaca
- Tweit RC, Finch DM (1994) Abert's Towhee (*Melospiza aberti*). In: Poole A (ed) *The birds of north America online*. Cornell Lab of Ornithology, Ithaca. Accessed 20 October 2013
- Wildenthal JL (1965) Structure of the primary song of the mockingbird (*Mimus polyglottos*). *Auk* 82:161–189
- Wiley RH (1991) Association of song properties with habitats for territorial oscine birds of eastern North America. *Am Nat* 138:973–993
- Wright TF, Dahlin CR, Salinas-Melgoza A. 2008. Stability and change in vocal dialects of the yellow-naped amazon. *Anim. Behav.* 76:1017-1027

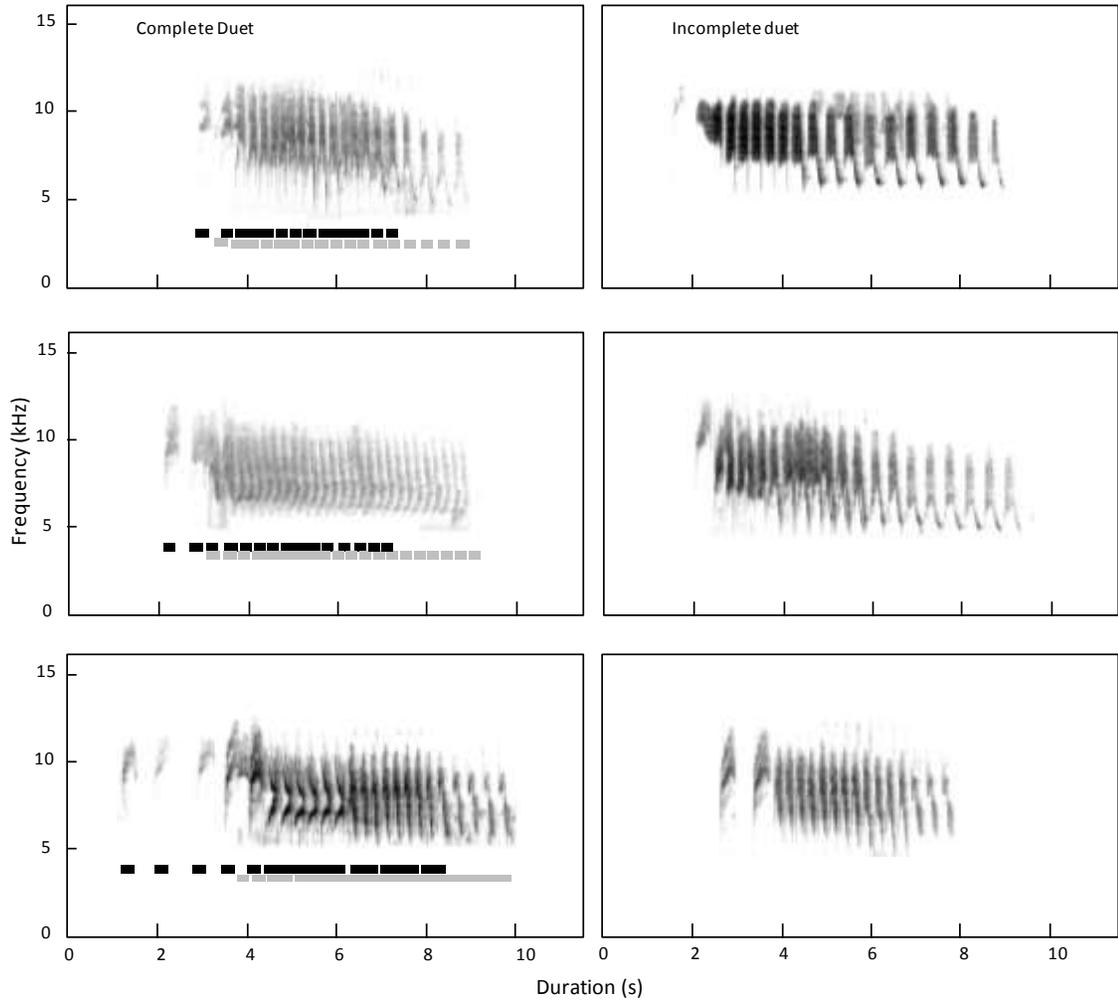
**Figures**



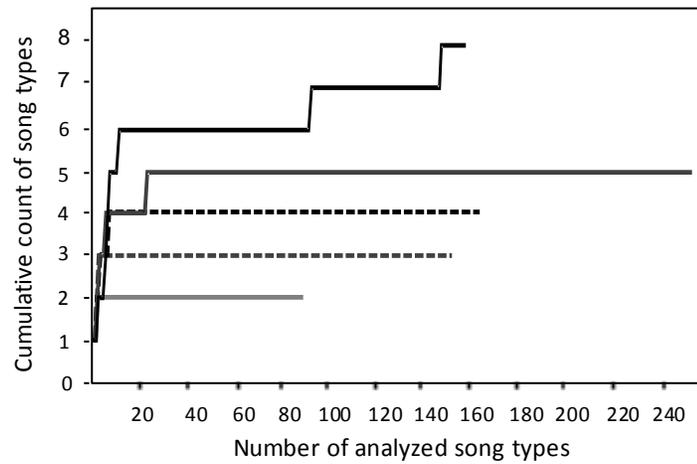
**Figure 2.1.** Sound spectrogram showing three examples of the two call types, *chip* and *tseet*, produced by both sexes of White-eared Ground-sparrows.



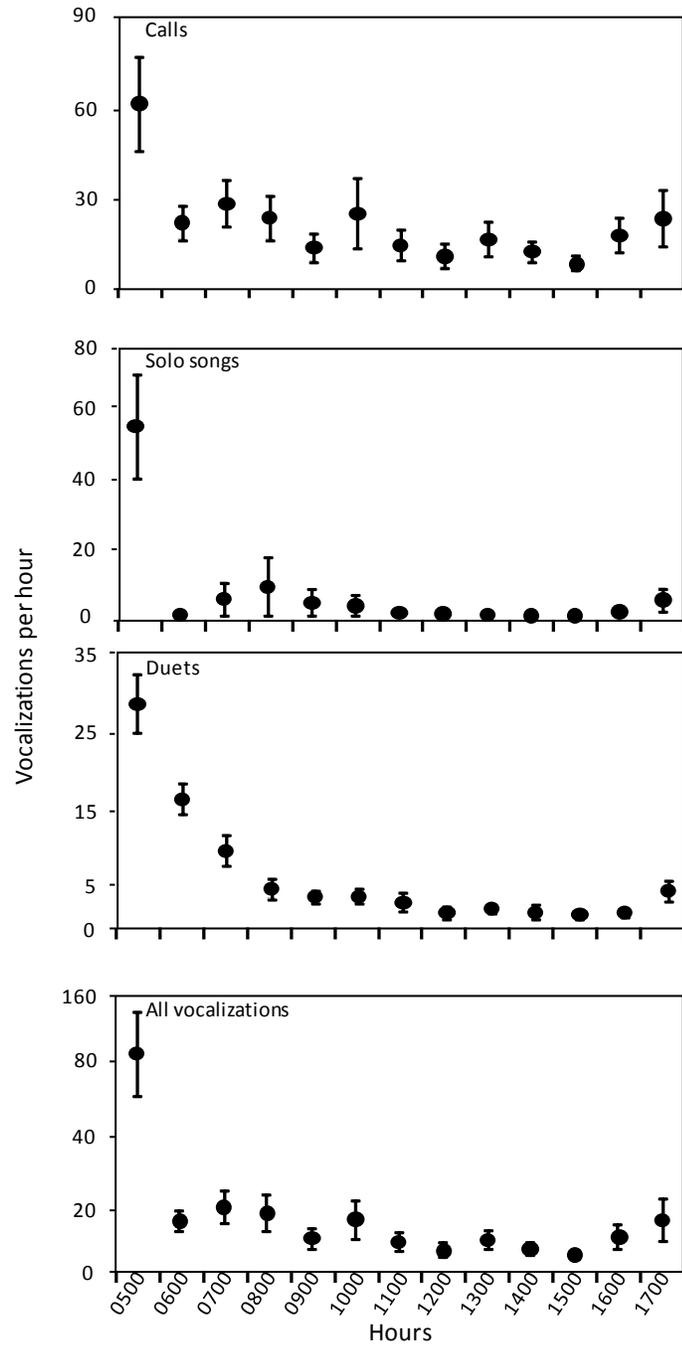
**Figure 2.2.** Sound spectrogram showing six common male solo songs in White-eared Ground-sparrows at four study sites in Costa Rica.



**Figure 2.3.** Sound spectrogram showing three examples of complete duets (produced by male and female of the pair) and three incomplete duets (duet contributions produced by a single individual with no response from the partner) of White-eared Ground-sparrows. Black and grey lines under complete duets represent the contribution of each individual to the duet.



**Figure 2.4.** Repertoire asymptote curve for 19 White-eared Ground-sparrows with 80 or more recorded songs, showing four males that reached an asymptote and one that did not. These five males were chosen to show the full range of variation in our dataset; the remaining 14 males had repertoire asymptote curves overlapped by the curves shown.



**Figure 2.5.** Diel pattern of White-eared Ground-sparrow vocalizations. Dots show means and the whiskers show standard error for  $n=14$  pairs recorded for a 507 hour period ( $36.2 \pm 16.3$  hours per pair). Vocalizations between 0450 and 0500 were included in the 0500-0600 period.

## Chapter 3

### **A quantitative description of vocalizations and vocal behaviour of Rusty-crowned Ground-sparrows (*Melospiza kieneri*)\***

---

\*This chapter is the outcome of joint research with D. Mennill

### Chapter summary

Several species of sparrow (Emberizidae) in the temperate zone provide model systems for understanding bird song and singing behaviour. In contrast, the vocal repertoire and vocal behaviour for most tropical sparrows is poorly understood, in spite of their impressive biodiversity. We present here the first detailed quantitative description of vocal repertoires and vocal behaviour of the Rusty-crowned Ground-sparrow (*Melospiza kieneri*), an endemic Mexican sparrow. We provide information on the effect of territory spacing on song repertoire-use and details of the diel pattern of variation in vocal output, using focal, autonomous, and opportunistic recordings in a population in El Tuito, Mexico. Our results demonstrate that Rusty-crowned Ground-sparrows produce three distinct categories of vocalizations—calls, solo songs, and duets—as in other *Melospiza* ground-sparrows. We found that solo songs and duets in this species showed acoustic structure intermediate to other northern and southern *Melospiza* ground-sparrows. Patterns of repertoire-use in male solo songs were highly similar between males holding nearby territories, suggesting that song learning may occur after territory establishment. The diel pattern for output of calls and solo songs showed a pronounced peak early in the morning, indicative of dawn-chorus singing behaviour. This study provides the first quantitative investigation of Rusty-crowned Ground-sparrow vocalizations, providing important descriptive information on this little-studied Neotropical *Melospiza* species.

## Introduction

The vocalizations and vocal behaviours of many species of north temperate sparrows (family: Emberizidae) have been studied in detail. Decades of investigations of temperate sparrows have led to the development of many key principles of avian acoustic communication that are widely accepted today (Marler 2004a, Catchpole and Slater 2008). For example, White-crowned Sparrows (*Zonotrichia leucophrys*) were the initial species used to examine geographic variation at both broad and local scales (Marler and Tamura 1962) which led to the study of cultural transmission in animals that learn their vocalizations (Marler 1970; 2004b). Song syntax was first studied in Swamp Sparrows (*Melospiza georgiana*), providing evidence that animals arrange their vocalizations with species-specific syntactical structure, much like human language (Marler and Pickert 1984). Our understanding of the production of overtones (Nowicki 1987), the function of graded signals (Beecher and Campbell 2005, Searcy and Beecher 2009), and the dynamics of conventional signals during aggressive interactions (Vehrencamp 2001) arise from seminal studies of Song Sparrows (*Melospiza melodia*).

Information on the vocal repertoires and vocal behaviour for the majority of tropical sparrows, in contrast, is limited or absent. The sole exception is the Rufous-collared Sparrow (*Zonotrichia capensis*); this widely studied tropical sparrow has been instrumental in enhancing our understanding of geographic variation and the relationship between habitat and population divergence in vocalizations (e.g. King 1972; Handford 1988; Handford and Loughheed 1991; Kopuchian et al. 2004; Danner et al. 2011). Further investigations of the vocalizations and vocal behaviour of other tropical sparrow species is an important research priority because such studies will allow us to conduct experimental studies to expand our understanding of animal vocal behaviour generally.

In this investigation we studied the Rusty-crowned Ground-sparrow (*Melospiza kieneri*). This endemic Mexican sparrow inhabits both deciduous and dry forest habitat from sea level to 2000 m, with a range extending from the south of Sonora to the northwest of Oaxaca, and the interior of Jalisco (Howell and Webb 1995; Rising 2011). Pairs of Rusty-crowned Ground-sparrows appear to defend territories using vocalizations, as do the other tropical species in the genus (Chapters 5 and 6), although their vocal repertoires and behaviour have never been described in detail (Howell and Webb 1995; Rising 2011). With this study our goal is to present the first quantitative description of the vocal repertoire and vocal behaviour of the Rusty-crowned Ground-sparrow. In addition, we investigate the relationship between territory spacing and song type sharing, and describe the diel pattern of variation in vocal output.

## **Methods**

### Field recordings

We recorded Rusty-crowned Ground-sparrows at Rancho Primavera, El Tuito, Jalisco state, Mexico (20°21'N, 105°20'W, 585 m a.s.l.). We collected recordings from 27 June to 3 July 2012, during the species' breeding season (Rowley 1962). Our field observations confirmed that some of the study birds were actively breeding during the recording period: for two pairs we observed adults carrying nesting material, and for two other pairs we observed behaviour consistent with incubation (i.e. one member of the pair disappeared after a period of foraging early during the day).

Recorded individuals were not colour-banded; we distinguished between territorial pairs based on their location (we collected GPS coordinates of the centre of each identified

territory). Based on previous field observations of this species, and observations of colour-banded study populations of a closely related species (White-eared Ground-sparrow, *Melospiza leucotis*; Chapters 2, 5, and 6), we estimated the territories of Rusty-crowned Ground-sparrows to have a diameter of 70-100 m. Therefore, paired birds that were > 50 m away from the centre of the neighbouring territory were considered to be a different pair. We monitored and confirmed the daily presence of previously recorded pairs at the same locations. Together, these observations made us confident that pairs recorded on different days were distinct and that pairs occupied the same territories throughout our recording period. The sexes are monomorphic in Rusty-crowned Ground-sparrows, so we were unable to assign sex to our unbanded study animals. In a sister species, however, our field observations of colour-banded pairs reveals that vocalizations are sex-specific (Chapter 2). We make the assumption that the same pattern is true in this species, and our field observations support this assumption (i.e. only one member of the pair produced some vocalizations, whereas others were produced by both members of the pair).

We used three recording techniques to describe the vocal and repertoire behaviour for this species. First, we collected focal recordings by following a territorial pair for 1 h period between 0700 and 0800 h (at the sun rises at approximately 0705 h during the studied period); each pair was recorded on one day using this technique (n = 13 pairs). Second, we used autonomous digital recorders (Wildlife Acoustics Song Meters model SM1) set up inside birds' territories to record for a period that varied from 6 to 14 hours (mean  $\pm$  SE:  $10.33 \pm 2.18$  h; n = 5 pairs). All of these recordings included a continuous period from 0700 to 0900 h, when this species is most vocally active (see Results). Third, we collected opportunistic recordings between 0800 and 1900 h while we were walking nearby or inside bird territories, when we detected birds vocalizing. Focal and opportunistic recording were conducted with a shotgun microphone

(Sennheiser ME66/K6) connected to a digital recorder (Marantz PMD660 and Marantz PMD661), and with a parabola-mounted omnidirectional microphone (23 in Telinga parabola with a Sennheiser ME62/K6 microphone) and digital recorder (Zoom H4n). All recordings were conducted in WAV format, with 16 bit accuracy and a 44.1 kHz sampling rate.

### Vocal analysis

We classified vocalizations based on their appearance on sound spectrograms, following similar approaches used in other studies (e.g. Franco and Slabbekoorn 2009, Odom and Mennill 2010). We measured the fine-structural details in Raven Pro 1.4 sound analysis software (Cornell Lab of Ornithology, Ithaca, NY, USA), using a combination of the waveform, spectrogram, and power spectrum to obtain the most accurate measurements (Charif et al. 2004, Redondo et al. 2013). We generated spectrograms with a temporal resolution of 5.8 ms and a frequency resolution of 188 Hz using the following settings: Hann window with 50 % overlap and 256 Hz transform size.

For each vocalization, we measured the duration (s), the minimum frequency (Hz), the maximum frequency (Hz), and the frequency of maximum amplitude (Hz). From all recordings, we calculated the number of unique solo song types produced per individual. We defined solo song types based on the number of different elements (smaller discrete unit of continuous sound) and the element shape. Each solo song type was then compared among others included in a library of reference developed for this species. We annotated the number of calls, solo songs, and duets produced by each pair from 0500 to 2000 hours to analyze diel variation in the vocal output, based on data from the autonomous recorders.

### Statistical analysis

We evaluated whether the fine structural details of two distinct call types (see Results) differed significantly from each other using a two sample t-test. We first calculated an average for each fine acoustic measurement per call type per pair, and then used these values as the dependent variable in the analysis, and the call type as the independent variable. We report exact *P*-values based in Bonferroni corrections ( $\alpha = 0.05$  divided by four) for multiple comparisons; four separate tests were conducted for each of the four measured acoustic characters.

We estimated male solo song repertoire size for all individuals where we recorded  $\geq 80$  songs, using the Wildenthal equation for curve-fitting (1965). We used a Markov chain analysis to estimate if the ground-sparrows delivered song types with a predictable order using the technique described in Lemon and Chatfield (1971). This approach analyzes the probability of singing a song type within the individual's repertoire as a function of the previous song type (Leonardo and Konishi 1999).

We conducted a Mantel test to evaluate repertoire-use similarity, measured using a Morisita index of similarity as described in Chapter 5, as a function of the physical distance between individuals' territories. We defined repertoire-use similarity as the production of shared vocalization types in similar proportion between males (see Chapter 5 for details). We predicted that closer individuals would have higher repertoire-use similarity than would individuals further away from each other.

To examine patterns of diel variation in vocal output within this species, we calculated the number of vocalizations per hour (mean  $\pm$  SE) from 0500 to 2000 hours. We then used Kolmogorov-Smirnov one-sample tests to compare if the average proportion of calls, solo songs and combined vocalizations (both calls and solo songs) showed a peak output during the day. We chose this nonparametric test because we had a small sample size and the data were not

normally distributed. The values of  $P$  for Mantel test and Kolmogorov-Smirnov test are reported based in 10000 permutations.

For duets, we only reported descriptive statistics because our sample size of this vocalization type was too small for statistical analysis. We conducted the statistical analysis in PAST (version 2.17; Øyvind Hammer, Natural History Museum, University of Oslo, Norway) and report means  $\pm$  SE throughout the paper.

## Results

The vocal repertoire of Rusty-crowned Ground-sparrows includes three main types of vocalizations: calls, solo songs, and duets. Calls ( $n = 956$  calls recorded from 12 pairs, one recorded pair never produced calls during our recording periods) and solo songs ( $n = 1906$  songs recorded from 12 males, one recorded male never produced songs during our recording periods) were common vocalizations. Duets, in contrast, were rare. We recorded only eight duets from three pairs over 137 hours of recordings.

We recorded calls from both members of the pair. We observed two types of calls which we distinguish as “*chip*” and “*tseet*” (Figure 3.1). These two calls were significantly different in their fine-structural characteristics (two sample  $t$ -test:  $p < 0.04$ ,  $n = 19$ , for all comparisons after Bonferroni correction). On average, the duration of the *chip* call was  $0.07 \pm 0.004$  s (range = 0.06 – 0.10 s, CV = 18.3 %), with a minimum frequency of  $7850 \pm 104$  Hz (range = 6968 – 8284 Hz, CV = 4.6 %), a maximum frequency of  $12,654 \pm 169$  Hz (range = 10,867 – 12,551 Hz, CV = 5.0 %), and a frequency of maximum amplitude of  $8574 \pm 79$  Hz (range = 7977 – 8964 Hz, CV = 3.2 %). We observed birds producing *chip* calls when the pair was foraging and when they were close to the nest during construction ( $n=2$  pairs).

On average, the duration of the *tseet* call was  $0.36 \pm 0.026$  s (range = 0.26 – 0.41 s, CV = 19.4 %), with a minimum frequency of  $7204 \pm 232$  Hz (range = 6596 – 8356 Hz, CV = 8.5 %), a maximum frequency of  $9478 \pm 245$  Hz (range = 8241 – 10,351 Hz, CV = 6.9 %), and a frequency of maximum amplitude of  $8050 \pm 193$  Hz (range = 7373 – 8958 Hz, CV = 6.3 %). We observed the ground-sparrows using this vocalization when pair members were far apart from each other.

We observed only one individual per pair producing solo songs, and we assumed that this was the male (an observation which matches our knowledge of other ground-sparrow species). Male solo songs were variable and readily classifiable into distinct song types (Figure 3.1). We detected 29 unique song types in our sample of 1482 analyzed songs. Male solo songs had three sections: (1) the introductory section began with high frequency elements similar to *chip* calls; (2) the middle section contained broadband frequency-modulated elements; and (3) the concluding section contained a trill (Figure 3.1). Solo songs showed an average duration of  $2.2 \pm 0.1$  s (range = 1.6 – 4.1 s, CV = 22.7 %), a minimum frequency of  $3470 \pm 121$  Hz (range = 2607 – 4916 Hz, coefficient of variation: CV = 18.8 %), a maximum frequency of  $11,111 \pm 130$  Hz (range = 8949 – 11918 Hz, CV = 6.3 %), and a frequency of maximum amplitude of  $5635 \pm 111$  Hz (range = 3728 – 6388 Hz, CV = 10.7 %).

Among 10 males for which we recorded  $\geq 80$  songs, the repertoire size varied from four to ten song types (average:  $6.3 \pm 0.7$  songs, Figure 3.2); all 10 males analyzed reached a repertoire asymptote. All of the individuals we studied delivered their song types in a predictable order that deviated significantly from random (Markov chain:  $p < 0.007$  for all individuals,  $n = 10$ ). Individuals with nearby territories showed more similar patterns of repertoire use than they did with far-away individuals (Mantel test:  $r = 0.49$ ,  $p = 0.002$ ,  $n = 10$ ).

Duets were produced by both members of the pair and included elements overlapping in

both time and frequency (Figure 3.1). Vocalizations used to construct a duet were different from vocalizations used as solo songs (Figure 3.1). Complete duets showed an average duration of  $4.3 \pm 0.4$  s (range = 3.7 – 5.0 s, CV = 15.8 %), minimum frequency of  $1314 \pm 35$  Hz (range = 1244 – 1349 Hz, CV = 4.6 %), maximum frequency of  $11,240 \pm 539$  Hz (range = 10,188 – 11,971 Hz, CV = 8.3 %), and frequency of maximum amplitude of  $7763 \pm 397$  Hz (range = 6977 – 8250 Hz, CV = 8.8 %). On three occasions, we observed just one individual of the pair producing an unanswered duet contribution, which was shorter in duration than a complete duet (Figure 3.1). We classified these vocalizations as “incomplete duets” (Figure 3.1). Incomplete duets showed an average duration of  $3.0 \pm 0.4$  s (range = 1.9 – 3.8 s, CV = 26.0 %), minimum frequency of  $4747 \pm 686$  Hz (range = 3277 – 6600 Hz, CV = 32.3 %), maximum frequency of  $10,215 \pm 367$  Hz (range = 9327 – 11,557 Hz, CV = 8.0 %), and frequency of maximum amplitude of  $8219 \pm 155$  Hz (range = 7666 – 8625 Hz, CV = 4.2 %).

Rusty-crowned Ground-sparrow vocal output varied throughout the day, with the highest peak occurring between 0700 and 1000 hours (KS:  $D_{\max} = 0.80$ ,  $p < 0.001$ ; Figure 3.3). With the 59 % of all vocalizations of the day occurred during this peak in vocal output (Figure 3.3). The same diel patterns for combined vocalizations was observed for both calls ( $D_{\max} = 0.72$ ,  $p < 0.001$ ; Figure 3.3), and solo songs ( $D_{\max} = 0.73$ ,  $p < 0.001$ ; Figure 3.3). Birds produced too few duets to conduct a meaningful analysis ( $n = 8$  duets from 3 pairs) although all duets were recorded throughout the morning (range: 0705h to 1216h).

## Discussion

The vocal repertoire of Rusty-crowned Ground-sparrows includes three main categories of vocalizations: calls, solo songs, and duets. Prior anecdotal descriptions of this species'

vocalizations mention only solo songs (Howell and Webb 1995; Rising 2011). Calls and duets were produced by both individuals of the pair, whereas solo songs were produced only by one individual in the pair; we assumed that the solo singers were males based on knowledge of vocal behaviour in related ground-sparrow species. Pair members create duets using vocalizations different from male solo songs, although duets were quite rare during our recording period. This ground-sparrow exhibited a diel pattern of variation with one peak of vocal output, coincident with dawn. This is the first quantitative description of the vocalizations of this little-studied endemic Mexican sparrow.

Rusty-crowned Ground-sparrows produce two types of calls, which we distinguish as *chip* and *tseet* calls. Two similar call types have been reported in White-eared Ground-sparrows (Chapter 2). Both species sharing similarity in acoustic structure and context of call use. As in White-eared Ground-sparrows, our observations of Rusty-crowned-ground-sparrows revealed that the *chip* call was used mainly in the context of contact between the pair members while foraging. We also observed birds producing this call when we were close to the nest site during the nest construction stage, which suggests this vocalization may also be used as an alarm signal (mobbing call), as has been suggested for White-eared Ground-sparrow *chip* calls (Chapter 6). The *tseet* calls also appear to be used to initiate or maintain contact, but less frequently than *chip* calls.

Both call types showed different acoustic structures that may affect the distance and position perception, as has been suggested for both call types in White-eared Ground-sparrows (Chapter 2). The *chip* call occurs at a higher frequency, with a broad bandwidth, and short duration, making it easy to localize the position of the sender (Marler 2004b), but these properties may reduce the distance that the call can travel throughout the habitat (Wiley 1991;

Bradbury and Vehrencamp 2011). In comparison, the *tseet* call showed narrow bandwidth, with a longer duration, suggesting that this vocalization can transmit over longer distances, but may be harder for potential receivers to localize as a result (Wiley 1991; Bradbury and Vehrencamp 2011). The acoustic properties of *chip* calls may help other individuals to localize potential predators, initiating a mobbing response, thereby better deterring potential predators (Marler 2004b; Sandoval and Wilson 2012).

Solo song was the most variable vocalization in terms of structure, because it included several types of elements in different combinations and frequency of occurrence, a pattern found widely among sparrows (Searcy 1992; Beecher et al. 2000). These high levels of variability are believed to be the result of sexual selection on this vocalization, by female selection or male-male competition (Searcy 1992; Gil and Gahr 2002). In the Rusty-crowned Ground-sparrow, only a single individual within a pair produced this vocalization; this was likely the male, based on studies in other species within this genus: Prevost's (*M. biarcuata*) and White-eared Ground-sparrows (Chapters 2, 5, and 6). In some sparrows, females prefer males with larger vocal repertoires (Searcy 1984, Reid et al. 2004). If this is true in Rusty-crowned Ground-sparrows, this may be a force driving the elaboration of male vocal repertoires. In other sparrows, males use solo song repertoires as a signal to male-male interactions (Beecher et al. 1994, Vehrencamp 2001). If this holds true in Rusty-crowned Ground-sparrows, then shared song types between neighbouring males may be an important social force that selects for vocal repertoires in solo song types.

We observed a relationship between repertoire-use similarity and distance between territories, where nearby males share and use their repertoires more similarly than with males located at further distances; this pattern has also been observed in a sister taxon, the White-

eared Ground-sparrow (Chapter 5). This pattern may indicate that males tend to learn their songs and use more of their repertoire during the territory establishment period (McGregor and Krebs 1989). Sharing repertoire characteristics with the neighbours is advantageous because it may help to reduce the territorial response of the neighbours (Fisher 1954), and also may facilitate male-male interactions (Beecher et al. 2000). Another advantage to repertoire sharing between immediate neighbours is the use of similar song types during male-male interactions, especially if matched song types are important signals of escalation during interactions (Krebs et al. 1981; Todt and Naguib 2000). To further evaluate these possible hypotheses for solo song function, future playback studies will be helpful.

In general, male solo song and duet structure in this ground-sparrow showed an intermediate pattern between the song and duet structures observed among northern and southern species in the genus. For example, in the northern species solo songs contain longer trills (Tweit and Finch 1994; Johnson and Haight 1996; Benedict et al. 2011) which are similar to several trill elements in the Rusty-crowned Ground-sparrows song (Fig. 3.1: song types 11 and 12); however, the southern species' songs are a combination of several elements including frequency-modulated elements and trills (Chapter 5 and 6), as are also observed in other Rusty-crowned Ground-sparrows solo songs (Fig. 3.1: song types 13 to 23). On the other hand, duets of Rusty-crowned Ground-sparrows included introductory elements similar to the introductory elements observed in the duets of Prevost's and White-eared Ground-sparrows (Chapters 2 and 6), but the elements of the main part of the duet (where the majority of the overlap occurred between the vocalization of both pair individuals) were similar to the elements observed in the duets of California Towhee (*M. crissalis*; Benedict and McEntee 2009).

The habitat structure where birds inhabit may influence the characteristics of vocalizations (Boncoraglio and Saino 2007). Rusty-crowned Ground-sparrows inhabit both deciduous and dry forests (Howell and Webb 1995; Rising 2011), habitats more open than those inhabited by the southern species (e.g., thickets and young successional forest), but closely resemble the habitats of northern species (e.g., desert vegetation and grasslands). Alternatively, the presence of the observed intermediate properties of solo songs in Rusty-crowned Ground-sparrow may be the result of phylogenetic relationships, given that this species shows similar relatedness with both ground-sparrow phylogenetic groups (DaCosta et al. 2009). A careful evaluation of habitat structure and phylogenetic effect is necessary to understand what influences this intermediate vocal pattern.

Duets were produced with a different vocalization than those used for solo songs, a pattern that appears to be widespread in *Melospiza* species (Benedict and McEntee 2009; Chapters 2, 6, and 7), but rare in many other duetting species where birds use the same vocalizations for solos and duets (e.g. Mann et al. 2003, Mennill and Vehrencamp 2005, Logue 2006). Although we obtained a limited number of duets during our recordings study, our observations suggest that duets in this species are used in a similar way that has been observed for the White-eared Ground-sparrow: territory defence and pair contact (Chapter 6). For example, when we played back duets to previous recorded pairs to corroborate their presence on their territory, both individuals approached quickly to the playback loudspeaker. We also observed duet production when one individual of the pair arrived next to the other. Future studies will require larger sample sizes and playback studies to corroborate and test these hypotheses.

Rusty-crowned Ground-sparrows showed significant variation in vocal output over the course of the day. The primary peak in vocal output occurred from 0700 to 1000, just after sunrise, coinciding with the dawn chorus in the study area. Calls and solo songs showed the same pattern throughout the day. A similar pattern has been observed in the White-eared Ground-sparrow (Chapter 2); although in general this species produced more vocalization per hour per individual throughout the day.

In conclusion, Rusty-crowned Ground-sparrows showed a repertoire size similar to that observed to the White-eared Ground-sparrows, although the vocalizations shared similar acoustic structural characteristics with both northern and southern species in this genus. The function of each category of vocalization is also similar to those reported previously for closely related species. Male solo song repertoire-use similarity was higher in nearby males, suggesting that song vocal learning may occur after territory establishment, as has been suggested for White-eared Ground-sparrows. The description of bird vocal repertoire and diel pattern is highly recommended because it is the basic information required to conduct comparative studies and address experimental questions.

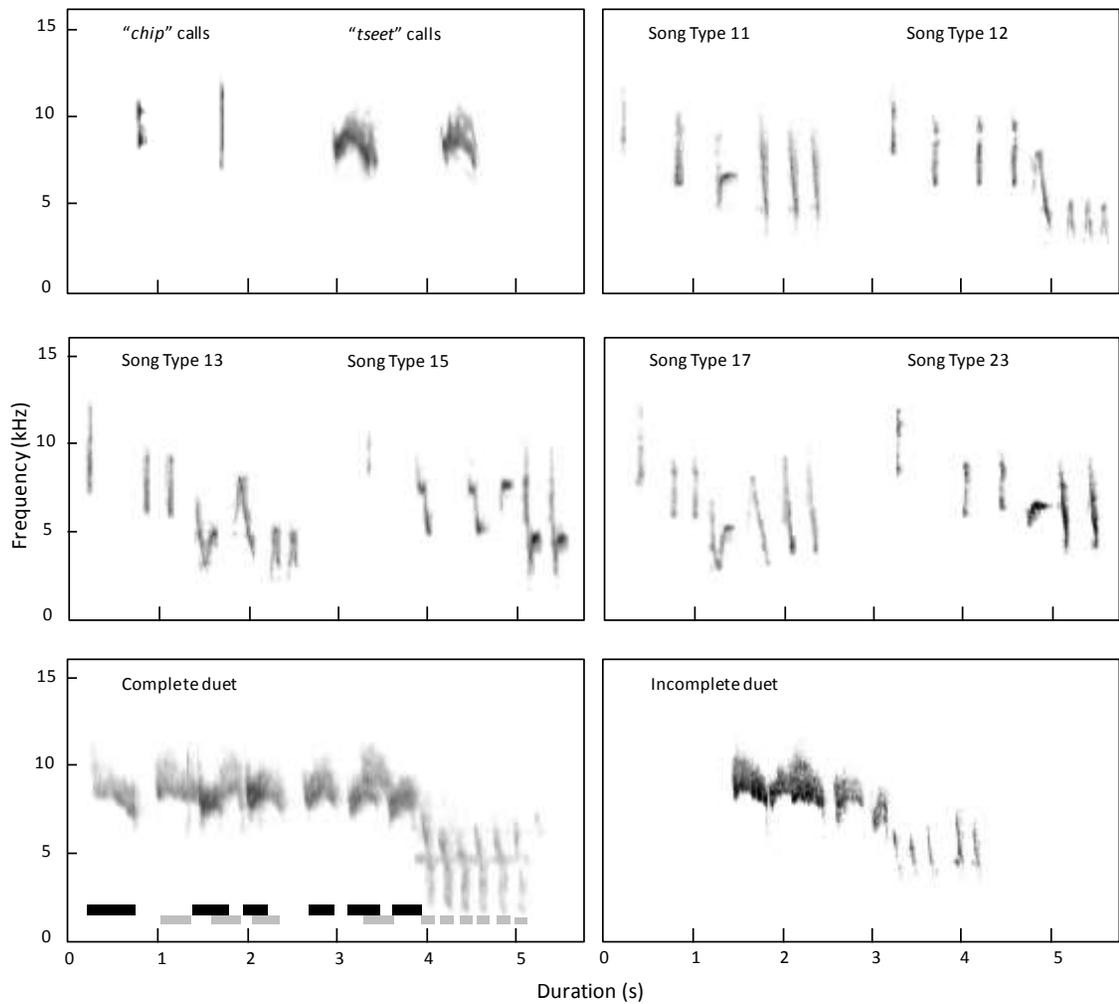
## References

- Beecher MD, Campbell SE. 2005. The role of unshared songs in singing interactions between neighbouring song sparrows. *Animal Behaviour* 70:1297–1304.
- Beecher MD, Campbell SE, Nordby JC. 2000. Territory tenure in song sparrows is related to song sharing with neighbours, but not to repertoire size. *Animal Behaviour* 59:29–37.
- Beecher MD, Campbell SE, Stoddard PK. 1994. Correlation of song learning and territory establishment strategies in the Song Sparrow. *Proceedings of the National Academy of Sciences* 91:1450–1454.
- Benedict L, McEntee JP. 2009. Context, structural variability and distinctiveness of California Towhee (*Pipilo crissalis*) vocal duets. *Ethology* 115:77–86.
- Benedict L, Kunzmann MR, Ellison K, Purcell KL, Johnson RR, Haight LT. 2011. California Towhee (*Melospiza crissalis*). In: Poole A, editor. *The birds of North America Online*. Ithaca (NY): Cornell Lab of Ornithology.
- 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.
- Bradbury JW, Vehrencamp SL. 2011. *Principles of animal communication*. 2nd ed. Sunderland (MA): Sinauer Associates.
- Catchpole CK, Slater PJB. 2008. *Bird song biological themes and variation*. Cambridge (NY): Cambridge University Press.
- Charif R, Clark C, Fristrup K. 2004. *Raven 1.2 User's Manual*. Ithaca (NY): Cornell Laboratory of Ornithology.
- DaCosta JM, Spellman GM, Escalante P, Klicka J. 2009. A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *Journal of Avian Biology* 40:206–216.
- Danner JE, Danner RM, Bonier F, Martin PR, Small TW, Moore IT. 2011. Female, but not male, tropical sparrows respond more strongly to the local song dialect: implications for population divergence. *The American Naturalist* 178:53–63.
- Fisher JB. 1954. Evolution and bird sociality. In: Huxley J, Hardy AC, Ford EB, editors. *Evolution as a Process*. London: Allen & Unwin.
- Franco R, Slabbekoorn H. 2009. Repertoire size and composition in great tits: a flexibility test using playbacks. *Animal Behaviour* 77:261–269.
- Gil D, Gahr M. 2002. The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology and Evolution* 17:133–141.
- Handford P. 1988. Trill rate dialects in the Rufous-collared Sparrow, *Zonotrichia capensis*, in northwestern Argentina. *Canadian Journal of Zoology* 66:2658–2670.
- Handford P, Loughheed SC. 1991. Variation in duration and frequency characters in the song of the Rufous-collared Sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects and body size. *Condor* 93:644–658.
- Howell SNG, Webb S. 1995. *A guide to the birds of Mexico and northern Central America*. New York (NY): Oxford University Press.

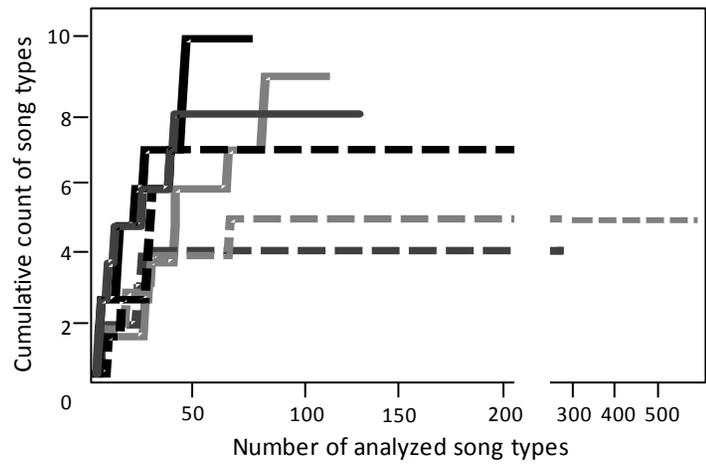
- Johnson RR, Haight LT. 1996. Canyon Towhee (*Melospiza fuscus*). In: Poole A, editor. The birds of North America Online. . Ithaca (NY): Cornell Lab of Ornithology.
- King JR. 1972. Variation in the song of the Rufous-Collared Sparrow, *Zonotrichia capensis*, in Northwestern Argentina. *Zeitschrift für Tierpsychologie* 30: 344–373.
- Kopuchian C, Lijtmaer DA, Tubaro PL, Handford P. 2004. Temporal stability and change in a microgeographical pattern of song variation in the Rufous-collared Sparrow. *Animal Behaviour* 68:551–559.
- Krebs JR, Ashcroft R, van Orsdol K. 1981. Song matching in the Great Tit, *Parus major* L. *Animal Behaviour* 29:918–923.
- Lemon RE, Chatfield C. 1971. Organization of song in cardinals. *Animal Behaviour* 19:1–17.
- Leonardo A, Konishi M. 1999. Decrystallization of adult birdsong by perturbation of auditory feedback. *Nature* 399: 466–470.
- Logue DM. 2006. The duet code of the female black-bellied wren. *Condor* 108:326-335.
- Mann NI, Marshall-Ball L, Slater PJB. 2003. The complex song duet of the Plain Wren. *Condor* 105:672-682.
- Marler P. 1970. A comparative approach to vocal learning: song development in White-crowned Sparrows. *Journal of Comparative and Physiological Psychology* 71:1–25.
- Marler, P. 2004a. Science and birdsong: the good old days. In: Marler P, Slabbekoorn H, editors. *Nature’s music, the science of bird song*. San Diego (CA): Elsevier Academic Press.
- Marler, P. 2004b. Bird calls: a cornucopia from communication. In: Marler P, Slabbekoorn H, editors. *Nature’s music, the science of bird song*. San Diego (CA): Elsevier Academic Press.
- Marler P, Pickert R. 1984. Species-universal microstructure in the learned song of the Swamp Sparrow, *Melospiza georgiana*. *Animal Behaviour* 32:673–689.
- Marler P, Tamura M. 1962. Song “dialects” in three populations of White-crowned Sparrows. *Condor* 64:368–377.
- McGregor PK, Krebs JR. 1989. Song learning in adult great tits (*Parus major*): effects of neighbours. *Behaviour* 108:139–159.
- Mennill DJ, Vehrencamp SL. 2005. Sex differences in the singing and duetting behavior of neotropical Rufous-and-white Wrens (*Thryothorus rufalbus*). *Auk* 122:175-186.
- Nowicki S. 1987. Vocal-tract resonances in oscine bird sound production: evidence from birdsongs in a helium atmosphere. *Nature* 325:53–55.
- Odom KJ, Mennill DJ. 2010. A quantitative description of Barred Owl vocalizations and vocal activity. *Condor* 112:549–560.
- Redondo P, Barrantes G, Sandoval L. 2013. Urban noise influences vocalization structure in the House Wren *Troglodytes aedon*. *Ibis* 155:621–625.
- Rising JD. 2011. Family Emberizidae (buntings and New World sparrows). In: del Hoyo J, Elliot A, Christie D, editors. *Handbook of the birds of the world, Volume 16 tanagers to New World blackbirds*. Barcelona: Lynx Edicions.
- Rowley JS. 1962. Nesting of the birds of Morelos, Mexico. *Condor* 64: 253–272.

- Sandoval L, Wilson, DR. 2012. Local predation pressure predicts the strength of mobbing responses in tropical birds. *Current Zoology* 58:781–790.
- Searcy WA. 1984. Song repertoire size and female preferences in Song Sparrows. *Behavioral Ecology and Sociobiology* 14:281–286.
- Searcy WA. 1992. Song repertoire and mate choice in birds. *American Zoologist* 32:71–80.
- Searcy WA, Beecher MD. 2009. Song as an aggressive signal in songbirds. *Animal Behaviour* 78: 1281–1292.
- Todt D, Naguib M. 2000. Vocal interactions in birds: the use of song as a model in communication. *Advances in the Study of Behaviour* 29:247–295.
- Tweit RC, Finch DM. 1994. Abert's Towhee (*Melospiza aberti*). In: Poole A, editor. *The birds of North America Online*. Ithaca (NY): Cornell Lab of Ornithology.
- Vehrencamp SL. 2001. Is song-type matching a conventional signal of aggressive intentions? *Proceedings of the Royal Society of London B*: 268: 1637–1642.
- Wildenthal JL. 1965. Structure in primary song of the Mockingbird (*Mimus polyglottos*). *Auk* 82:161–189.
- Wiley RH. 1991. Association of song properties with habitats for territorial oscine birds of eastern North America. *American Naturalist* 138:973–993.

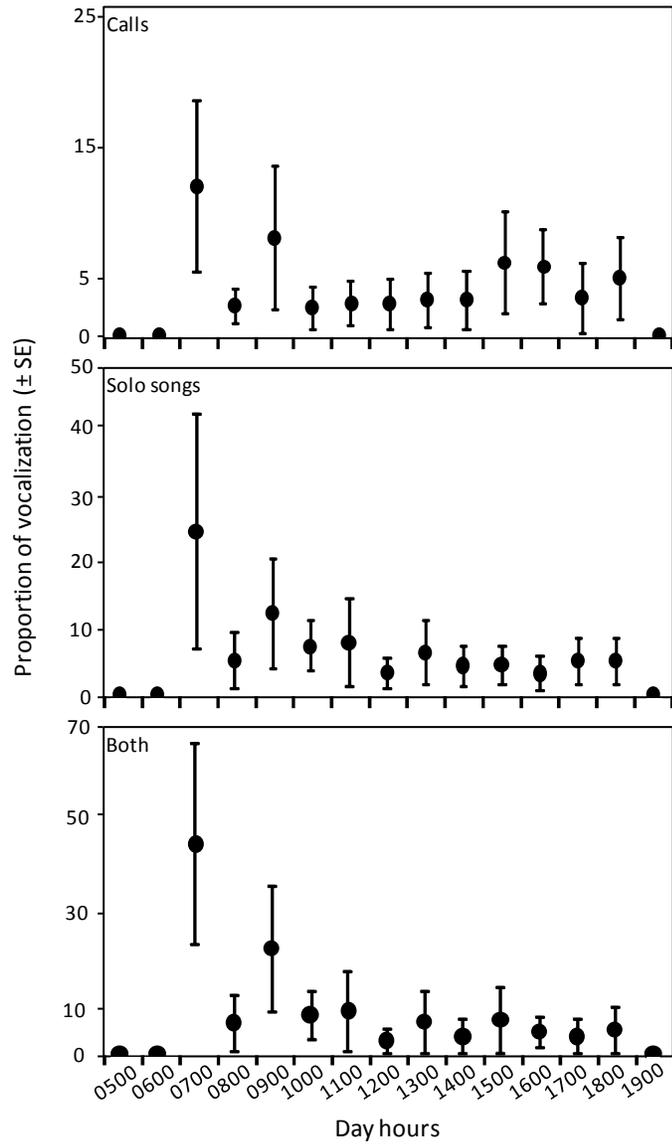
Figures



**Figure 3.1.** Sound spectrograms of the three main categories of vocalizations produced by Rusty-crowned Ground-sparrows, with two examples of the two call types; six common male solo song types produced in the study site; a complete duet (black and grey blocks show the contribution of each individual of the pair); and an incomplete duet featuring a contribution by one individual.



**Figure 3.2.** Repertoire asymptote curves for Rusty-crowned Ground-sparrows, showing six males that reached an asymptote. These six males were chosen to show the full range of variation in our dataset; the remaining four males had repertoire asymptote curves overlapped by the curves shown.



**Figure 3.3.** Diel variation in vocal output in Rusty-crowned Ground-sparrows according to calls, solo songs, and both vocalizations together. Dots show means and the whiskers show standard error for n= 5 pairs recorded for a 52 hour period.

## **Chapter 4**

**Transmission characteristics of solo songs and duets in neotropical thicket**

**habitat specialist bird\***

---

\*This chapter is the outcome of joint research with T. Dabelsteen and D. Mennill

### Chapter summary

The Acoustic Adaptation Hypothesis proposes that the structure of animal vocalizations is heavily influenced by habitat characteristics, and that animals will develop vocalizations and display behaviours that optimise the transmission properties of these signals. White-eared Ground-sparrows (*Melospiza leucotis*) live in early succession habitats with dense vegetation (thickets) where vocal communication is an ideal mode of communication for territory defence and mate attraction. Based on the Acoustic Adaptation Hypothesis, if both vocalizations are used in long distance communication, we expected that the solo songs and duets produced by ground-sparrows would exhibit structures that enhance sound transmission in thicket habitats. We conducted a sound transmission experiment where we broadcast and re-recorded solo songs and duets to study their transmission properties. We used two speaker heights and two microphone heights to simulate different perch heights of signalers and receivers, and four distances between the speakers and microphones to simulate variable distances of separation. We found that both solo songs and duets show similar patterns of degradation and attenuation with distance and with proximity to the ground. This result suggests that both solos and duets facilitate communication with receivers at similar distances. The highest perches, for both signalers and receivers, maximized acoustic transmission. This is the first study that evaluates the transmission properties of both songs and duets in birds, despite the fact that many bird species in the tropical forest produce both types of vocalizations. Surprisingly, we found that both solos and duets degraded to very low levels in less than a typical territory's diameter, suggesting that this species has not experienced strong selection for long distance communication.

## **Introduction**

The structure of vegetation and the ambient noise characteristics of wilderness habitats have a heavy influence on the structure of vocalizations produced by animals (e.g. Dabelsteen et al., 1993; Forrest, 1994; Balsby et al., 2003). Numerous investigations have demonstrated that animal signals are acoustically adapted to optimize transmission characteristics in their habitat, (Boncoraglio & Saino, 2007; Ey & Fisher, 2009). The structure of the vocalizations may also change over time, if habitat characteristics change, to enhance transmission distance (e.g. Perla & Slobodchikoff, 2002; Derryberry, 2009). By studying the transmission properties of animal vocalizations, we can explore the relationship between animal communication and animal habitats, and thereby the evolution of animal behaviour.

Some habitats may present more significant challenges for the transmission of animal vocalizations than others. In particular, noisy environments may present substantial communication challenges to both signallers and receivers, such as habitats near moving water or urban areas (Slabbekoorn, 2004; Redondo et al., 2013) or habitats with very dense vegetation (Slabbekoorn et al., 2002). In tropical environments, early successional habitats with dense vegetation—known as thickets—may present special barriers to signal transmission because vegetation causes scattering, reflection, and reverberation, thereby attenuating signals especially of high frequencies (Slabbekoorn et al., 2002; Dingle et al., 2008). Many of these habitats are also located close to noisy places such as river edges, streets, and towns (Sánchez-Asofeifa et al., 2001; Harvey et al., 2008; Biamonte et al., 2011), which may further impede acoustic communication of animals living therein (Ryan & Brenowitz, 1985; Slabbekoorn & Peet, 2003; Barker, 2008).

Studies of sound transmission have focused on the breeding vocalizations produced by animals, including a heavy focus on male songs (Boncoraglio & Saino, 2007; Ey & Fisher, 2009), probably because these vocalizations are among the most conspicuous long distance vocalizations used to attract females and deter territorial rivals (Andersson, 1994; Catchpole & Slater, 2008). According to the Acoustic Adaptation Hypothesis (Morton, 1975; Hansen, 1979), the acoustic characteristics of animal vocalizations are adapted to the habitat where they are typically transmitted (Boncoraglio & Saino, 2007; Ey & Fisher, 2009); several investigations of the transmission properties of bird songs confirm that this is the case (Ryan et al., 1990; Brown, et al. 1995; Sabatini et al., 2011). Yet animals also produce a wide variety of other acoustic signals beyond male breeding songs, including female songs, calls from both sexes, and vocal duets (Langmore, 1998; Matrosova et al., 2011; Geissmann, 2002; Marler, 2004). These vocalizations may also be used in long distance communication, and therefore may be acoustically adapted to their environment. We have a poor understanding of the transmission properties of other types of vocalizations in comparison to the breeding songs of males, and it is worthwhile to explore the acoustic adaptation of these other types of signals.

Our main objective in this investigation is to compare the transmission characteristics of the solo songs and duets of White-eared Ground-sparrows (*Melospiza leucotis*). This species specializes in dense thicket habitats of the Neotropics (Sandoval & Mennill, 2012), and males and females of this species live as territorial pairs throughout the year, as do many tropical birds (Stutchbury & Morton, 2008). White-eared Ground-sparrows produce three main types of vocalizations: both sexes produce quiet calls; males produce solo songs; and breeding partners combine their vocalizations to produce vocal duets (Chapters 2 and 6). Whereas some birds use the same vocalization for solos and duets, the duets of White-eared Ground-sparrows are

created with very different vocalizations than those used by males as solo songs (Chapter 2 and 5). Whereas male solo songs are frequency-modulated tones and at frequencies that vary from 3.5 to 11.2 kHz, the vocalizations males and females contribute to duets are rapid, noisy, and with frequencies that vary from 5.1 to 11.5 kHz (Chapter 2). According to the Acoustic Adaptation Hypothesis, it is reasonable to predict that White-eared Ground-sparrow solo songs for example, have evolved to enhance sound transmission through thicket habitats, especially because appear to be used for long distance communication (Chapter 2). Our field observations suggest that male songs are used mainly to attract females, and are produced from perches that vary between 1 and 3 m height on average. Duets, on the other hand, appear to be used for within-pair communication and for territory defence against other pairs and are produced mainly from perches close to or directly on the ground (unpub. data). The sound spectrograms of White-eared Ground-sparrow vocalizations, however, show unexpected patterns. Their vocalizations have broad bandwidth, relatively short duration of elements, high minimum frequency, and prominent trills. The Acoustic Adaptation Hypothesis predicts that vocalizations with narrow bandwidth, long duration, and with a low minimum frequency should maximize transmission in dense vegetation, and trills should be favoured in open environments, rather than in dense vegetation (Morton, 1975; Hansen, 1979, Boncoraglio & Saino, 2007; Ey & Fisher, 2009). A field-study of the transmission properties offers the opportunity to understand if these patterns could be an adaptation for optimizing communication range. We conducted a sound transmission experiment to evaluate the transmission characteristics of White-eared Ground-sparrow solo songs and duets. Specifically we addressed two questions: (1) Do the solo songs and duets of White-eared Ground-sparrows have different transmission properties? (2) Do transmission properties of solo songs and duets vary with the perch height used by the signaler or receiver? If White-eared Ground-sparrow songs and duets are used to communicate with

receivers at similar distances, we predicted that both types of vocalizations would share the same pattern of degradation and attenuation through thicket habitats. If one vocalization is used mainly for short-range communication (e.g., between pair members) and the other for long-range communication (e.g., with animals in adjacent territories), we predicted that one vocalization would show more degradation and attenuation than the other. Finally, we predicted that higher perches would increase sound transmission, as has been reported in other studies (Krams, 2001; Barker & Mennill, 2009; Barker et al., 2009); therefore, vocalizations should show higher levels of degradation and attenuation closer to the ground.

## **Methods**

### Study sites and territory measurements

We conducted this study in the Getsemaní region of Heredia province, Costa Rica (10°01'N, 84°06'W; 1300 m elevation), where White-eared Ground-sparrows are common inhabitants in young secondary forest edges, shade coffee plantations, and naturally occurring thickets. The study was conducted from 30 July to 2 August 2012, during the last part of this species' breeding season (Sandoval & Mennill, 2012). All playback sessions took place inside three typical territories of White-eared Ground-sparrows (one in a shade coffee plantation and two in natural thickets). All experiments took place between 6:00 and 9:00 h, a time when both male solo songs and vocal duets are commonly heard from this species (Chapter 2).

To describe vegetation density within the territories occupied by the study species, we measured the number of trees (plants > 2 m tall and with a diameter at breast height  $\geq$  10 cm), bushes (plants 1 - 2 m tall with the main trunk diameter of 2 - 10 cm), and the percentage of

ground covered by grasses and small plants (15 - 100 cm tall), in 19 White-eared Ground-sparrow territories. We collected 8 to 12 measurements per territory using a 2 × 2 m plots. We originally endeavoured to take 12 measurements in each territory, but some territories were too small for 12 plots; in other territories the land structure, including steep slopes, or creeks, prohibited 12 plots. We distributed the plots along the cardinal points at three distances from the territory centre: 5, 10, and 20 m.

#### Transmission playback stimuli

To create stimuli for playback, we used vocalizations recorded during previous investigations of this species. Recordings were collected with a Marantz PMD 661 digital recorder (sampling rate: 44.1 kHz; accuracy: 16-bit; file format: WAVE), and a Sennheiser ME66/K6 directional microphone. We selected our highest quality recordings, focusing on sounds with little or no overlapping background sounds and with a high signal-to-noise ratio for both male solo songs and duets (Figure 4.1). Sounds used in the experiment were selected from five different individuals and were representative of the species' repertoire. For male solo song stimuli, we chose a solo song from two males. For duet stimuli, we chose three duet contributions, one from a male and two from individuals of unknown sex (due to the dense vegetation at our study site, and the fact that pair members often forage in very close proximity, we could not assign the sex of the singer with confidence). We used duet recordings where we recorded non-overlapping duet contributions (i.e. incomplete duets, see Chapter 2), rather than the overlapping male-female contributions that are typical of this species' duets (i.e. a vocalization produced by both members of the pair singing simultaneously), because male and females overlap in frequency

and time (see Figure 1.3 in Chapter 2), making it impossible to separate the elements for the analysis.

We played entire solo songs ( $N = 2$ ) and duets ( $N = 3$ ), as well as isolated elements of solo songs ( $N = 6$  elements, 3 from each of 2 males' songs) and duets ( $N = 4$  elements from three different birds; Figure 4.1). The stimuli were composed of a sequence of five repetitions of two complete solo songs, three duet songs, and the ten elements. Each repetition was separated 3 s of silence. Solo songs, duets, and the separate elements were separated by 1.5 s of silence. For each solo song we selected three elements: one introductory element (I), one middle element (M), and one trill element (T, Figure 4.1). We selected four duet elements (D), in two duets we selected one element per duet, and in one duet we selected two elements (Figure 4.1).

Given the variable frequency range of solo and duet songs, we used different filters to isolate the sounds of interests, by excluding background sounds, for our playback stimuli. For solo songs and solo song elements we used the following filters: solo song 1: 1.5 – 11 kHz; solo song 2: 4- 13.5 kHz; element I1: 7.5 – 13 kHz; element I2: 4 – 11.5 kHz; element M1: 1.7 – 8 kHz; element M2: 4 – 9 kHz; element T1: 4 – 9.5 kHz; and element T2: 4 – 9 kHz (Figure 4.1). For duet songs and duet song elements we used the following filters: duet 1: 4 – 11.5 kHz; duet 2: 4 – 12 kHz; duet 3: 4 – 10.5 kHz; element D1: 6 – 12 kHz; element D2: 4 – 11 kHz; element D3: 6 – 12; element D4: 5 – 11 kHz (Figure 4.1). We applied these filters using the passive option of the Fast Fourier Transformed filter in Audition 1.0 (Adobe Systems, San Jose, CA, USA). Stimuli were standardized to -1 dB in Audition. The stimuli were transferred to a portable audio player (model: Ipod Touch Nano, Apple, Cupertino, CA) for playback in the field.

### Transmission experiment

We broadcast the stimuli from an active loudspeaker (Anchor Audio; Minivox; frequency response: 0.1 – 12 kHz), and re-recorded them using an omnidirectional microphone (Sennheiser ME62/K6) and a solid-state digital recorder (Marantz PMD661; sampling rate: 44.1 kHz; accuracy: 16-bit; file format: WAVE), connected via a microphone preamplifier (Sound Device MP-1; frequency response: 0.02 – 22 kHz). We played back the stimuli at a constant volume of 80 dB SPL, measured at 1 m from the speaker using a digital sound level meter (Radio Shack model 33-2055 using C weighting, slow response). As the distance between the loudspeaker and the microphone increased, we adjusted the level of our preamplifier so that we could still record the playback sounds. We always used a gain of 18 dB at 16 and 32 m of distance between the loudspeaker and microphone. Our broadcast amplitude, 80 dB SPL at 1m, matched how loud the ground-sparrow solo songs and duets are in the field according to the perception of two investigators with three years of experience in recording the study species.

For each of the three transmission tests, we played sounds across four horizontal distances (4, 8, 16, and 32 m between loudspeaker and microphone) and two microphones and speaker heights (0.4 and 2.2 m). We used these heights for the microphone and speaker to represent the two common heights where we have observed White-eared Ground-sparrows producing solo songs (i.e. higher height) and duets (i.e. lower height). The horizontal distances were selected to represent the distances we often observed between the pair members (i.e. the two shorter distances) and between neighbouring pairs (i.e. the two longer distances). Rather than repeating the playback at the four horizontal distances along a linear transect, as has been done in previous studies (e.g. Barker et al. 2009, Sabatini et al. 2011), we chose instead to playback sounds along four different axes within each territory, to look at the effect of a larger

and hence more representative part of the territories. We chose these playback axes according to the cardinal points in two territories, and in one territory the four transects started at the same point but they were distributed at different directions close to the south because the shape of the territory prevented us from conducting the transmission test in the cardinal directions. We measured the temperature (mean  $\pm$  SE:  $24.64 \pm 0.61$  °C) and relative humidity (mean  $\pm$  SE:  $94.78 \pm 0.17$  %) every 5 minutes during the experiment using the internal humidity and temperature device of the SM2+ Wildlife Acoustic Song Meters (Wildlife Acoustics Inc., Concord, MA, USA) placed at a height of 1 m inside each territory.

#### Sound analysis

We used SigPro 3.25 software (Pedersen, 1998), to analyze the re-recorded sounds. Rather than comparing the re-recorded sounds to the playback stimuli, we compared them to re-recorded sounds collected at a distance of 1.0 m. This allowed us to control for changes in the sound that may have arisen because of the playback equipment. For the 1.0 m recording, the speaker was oriented upwards and the microphone was hung 1.0 m directly overtop in the centre of an open field of 20  $\times$  20 m; we did this to avoid recording the re-recorded sound with reverberations produced by the ground and vegetation in the recording. The first three repetitions of each sound that were not overlapped by any other sound were selected for use in the analysis.

We compensated for the stationary background noise that contributes to the amplitude values of the experimental sounds. We measured the noise immediately before the start of the stimulus for each analyzed sound. As in other transmission studies (e.g. Sabatini et al., 2011), we assumed that the background noise before each stimulus was the same as the noise that

overlapped the experimental sounds. A detailed explanation of the estimation of noise values can be found in Sabatini et al. (2011).

For each experimental sound we measured the following four variables: the signal-to-noise ratio (the comparison between the amount of energy in the observed sound versus energy in the background noise immediately prior to the sound of interest), tail-to-signal ratio (the amount of energy in the reverberant tail compared with the energy in the observed sound), the blur ratio (the frequency-dependent attenuation and temporal distortion of the signal), and excess attenuation (attenuation beyond the spherical spreading of 6dB per doubling of the distance). Details about the formulas used to collect these measurements in SigPro are presented in Dabelsteen et al. (1993), Holland et al. (1998), and Lampe et al. (2007). For several of the 32 m playback sessions, the re-recorded sound was too faint for analysis, even with the use of the pre-amplifier.

#### Statistical analysis

We performed two general linear models (GLM) to analyze the effect of the sound transmission experiments on signal degradation. The first GLM was used to compare the transmission of entire solo songs versus entire duets, and the second one was used to compare the transmission of solo song and duet elements. We used as independent variables in the GLM the distance between the speaker and microphone (four levels), the speaker height (two levels), the microphone height (two levels), and stimulus (five levels for solo songs and duets, and ten levels for elements). The response variables were the four sound degradation measurements (signal-to-noise ratio, tail-to-signal ratio, the blur ratio, and excess attenuation) which we ran separately

in four independent models. We estimated only main effects and two-factor interactions in our analysis. Finally, we performed post hoc tests, conducting all pairwise comparisons between main effects and two-factor interactions using Bonferroni corrections. Our response variables were normally distributed (Kolmogorov-Smirnov normality test:  $p > 0.05$ ) and showed equality of variances.

Following by the technique used by several authors (Nemeth et al., 2001; Barker et al., 2009; Sabatini et al., 2011) we analyzed variation in background noise level by conducting another GLM in the region of the sound spectrum that remained after the filters were applied in each sound. We used as independent factors in the GLM the distance between the speaker and microphone (four levels), the speaker heights (two levels), the microphone heights (two levels), and sounds (five levels for solo songs and duets, and ten levels for elements). The response variable was the background noise level measurement.

Throughout, we report all values as mean  $\pm$  SE. Statistical analyses were conducted in JMP (version 10.0; SAS Institute, Cary, NC, U.S.A.).

## Results

### Vegetation characteristics

In the 19 analyzed White-eared Ground-sparrows territories we found 0.10 trees/m<sup>2</sup> (range: 0 – 0.23 trees/m<sup>2</sup>) and 0.45 bushes/m<sup>2</sup> (range: 0.06 – 1.20 bushes/m<sup>2</sup>). The percent cover of grass and small plants was 52% (range: 11.25 – 100%).

### Degradation of complete solo songs and duets

For comparisons between complete solo songs and duets, we observed several significant patterns for the main effects and two-factor interactions (Table 4.1). As distance increased, sounds showed lower signal-to-noise ratios, longer tail-to-signal ratios, a higher blur ratio, and increased excess attenuation, as expected (Table 2). When speakers were closer to the ground (0.4 m vs 2.2 m), sounds showed lower signal-to-noise ratio (Figure 4.2) but the other three variables were not statistically different. When microphones were closer to the ground (0.4 m vs 2.2 m), sounds showed lower signal-to-noise ratio, higher blur ratio, and increased excess attenuation (Figure 4.2). Solo songs showed lower signal-to-noise ratio and shorter tail-to-signal ratio than duets (Figure 4.3).

Less than the half of the interactions showed a significant effect in our models, and these interactions included distances, and speaker and microphone heights (Table 4.1). Distance  $\times$  speaker height and distance  $\times$  microphone height interactions showed higher signal-to-noise ratio, shorter tail-to-signal ratio, lower blur ratio, and decreased excess attenuation at shorter distances and higher perches (Table 4.1; Figure 4.4). Distance  $\times$  sound interaction showed longer tail-to-song ratio when increased the distance, and at all distances duets showed shorter tail-to-signal ratio than solo songs (Figure 4.4). Speaker height  $\times$  microphone height interaction showed lower signal-to-noise ratio and increased excess attenuation at lower heights (close to the ground); but longer tail-to-signal ratio at diagonal propagation (Figure 4.4). The remaining interactions were not significant (Table 4.1).

### Degradation of solo songs and duet elements

In addition to our analyses of entire solo songs and duets, we analyzed separately the elements that make up solo songs and duets. As with entire solo songs and duets, we found significant effects in signal-to-noise ratio, tail-to-signal ratio, blur ratio, and excess attenuation and several two-factor interactions (Table 4.3). As distance increased, elements showed lower signal-to-noise ratios, longer tail-to-signal ratios, a higher blur ratio, and increased excess attenuation, as expected (Table 4.2). When the speaker was closer to the ground (0.4 m), elements showed lower signal-to-noise ratio and increased excess attenuation. When the microphone was closer to the ground (0.4 m), elements showed lower signal-to-noise ratios, longer tail-to-signal ratios, a higher blur ratio, and increased excess attenuation. We failed to find any significant pattern of degradation on solo song and duet elements, and degradation depended specifically on the characteristics of each element (Figure 4.3).

More than the half of the interaction terms showed some effect in our analysis of solo song and duet elements, and these interactions included distance, and speaker and microphone heights (Table 4.3). Distance  $\times$  speaker height, distance  $\times$  microphone height, and distance  $\times$  element interactions showed higher signal-to-noise ratio, shorter tail-to-signal ratio, lower blur ratio, and decreased excess attenuation at shorter distances and higher perches (Table 4.3). Some elements degraded equally at 4 and 8 m; while others had higher degradation at 8 m. The degradation at 32 m was highest for all elements than at closer distances. Speaker height  $\times$  microphones height interactions showed lower signal-to-noise ratio and increased excess attenuation close to the ground, but longer tail-to-signal ratio and higher blur ratio at diagonal propagation (i.e. between high speakers and low microphones, or vice versa). Microphone

height  $\times$  element interaction showed a higher blur ratio at lower heights. The remaining interactions were not significant (Table 4.3).

#### Background noise variation

In our analysis of the background noise that preceded each bout of recording, we found that background noise levels varied with distance (Table 4.4), where there was slightly more background noise at 32 m, than at 16 m, and with similar noise levels at both 8 and 4 m. This result is likely caused by noise produced by an increase in the vegetation between the speaker and microphone with the distance, consequently wind will rustle a large number of leaves producing more background noise. The only interactions that affected the background noise levels were distance  $\times$  sound and speaker heights  $\times$  microphone heights (Table 4.4). Distance  $\times$  sound interaction showed more background noise at 32 m than at closer distances, probably due to these same causes. Distance  $\times$  speaker height interactions showed more background noise at lower heights (close to the ground), and this effect is likely caused by noise produced by wind rustling leaves in the dense understory.

#### Discussion

Using a transmission experiment, where we played the solo songs and duets of White-eared Ground-sparrows across several different distances and at two different speaker and microphone heights in this species' native thicket habitat, we showed that the degradation and attenuation of complete solo songs, duets, and their elements increased with distance and proximity to the ground. We found that solos and duets experienced similar patterns of

attenuation and degradation, indicating that both types of vocalizations transmit similar distances, and suggesting that both solos and duets are designed to communicate with potential receivers located at similar distances from signallers. Speaker and microphone height positively influenced the transmission of vocalizations, demonstrating that ground-sparrow solos and duets experienced less degradation and attenuation from higher perches. Patterns of attenuation were influenced by the interaction between the distance with other factors such as speaker and microphone height, and rarely with the type of sound analyzed.

The thicket habitats occupied by White-eared Ground-sparrows impose a limitation on visual communication due to the high density of vegetation; therefore acoustic communication may be an especially important modality for long-range signalling in thicket habitats. High vegetation density, however, affects sound transmission by increasing degradation (Nemeth et al., 2001; Slabbekoorn & Smith, 2002; Slabbekoorn, 2004), especially if the vocalizations are not adapted to transmit well in this type of habitat. Solo song elements with narrow bandwidth and long duration tend to transmit well in dense vegetation, but broadband, short elements do not (Wiley, 1991). Our results reveal that the solo songs and duets of White-eared Ground-sparrows are not well adapted to transmit in dense habitat. The measurements we collected of signal attenuation and degradation (signal-to-noise ratio, excess attenuation, and blur ratio) were higher than reported in other transmission studies. For example, in temperate forests, Common Blackbirds (*Turdus merula*; Dabelsteen et al., 1993) and Blackcaps (*Silvia atricapilla*; Mathevon et al., 2005) showed signal-to-noise ratios that were more than double of those report here, excess attenuation values were less than one third of our reported values, and blur ratio values were less than half of those reported here at longest distances. In one of the few studies of degradation conducted in tropical forest, Rufous-and-white Wrens (*Thryophilus rufalbus*; Barker

et al., 2009) showed signal-to-noise ratios that were 1.5 times higher than those reported here, excess attenuation values were less than one seventh of those report here, and blur ratio values were less than half of those reported here. These comparisons suggest that thicket habitats impose a significant barrier to effective communication and demonstrate that White-eared Ground-sparrow songs and duets – vocalizations with broad bandwidth, short duration, and repeated trill elements – are poorly adapted to transmit long distances inside thicket habitats. . Although solo songs and duets have different main functions in this species (Chapter 6), similarity in degradation may be the result of constrains that produce both vocalizations to evolve in the same way, or avoid the divergence in the acoustic characteristics for each vocalization in relationship to their main function. Another possible cause for the similarity in degradation between both vocalizations is that the potential receiver for both vocalizations (e.g., neighbour females and neighbour pairs) is allocated in a similar distance.

Field observations suggest that White-eared Ground-sparrow territories have a diameter of approximately 50 to 70 m (estimated territory sizes based on tracking 42 banded pairs over the last three years), and that birds often occupy territories that abut multiple neighbours (Chapter 5). Given our observations of the birds' territory sizes, combined with the rapid degradation reported here, solo songs and duets of these species are not expected to propagate more than one territory diameter, limiting the vocal interactions with other pairs or potential mates further than one territory apart.

White-eared Ground-sparrows may use behavioural strategies to enhance sound transmission, as has been reported for other bird species (e.g. Krams, 2001; Mathevon et al., 2005; Barker & Mennill, 2009). For example, we have observed birds singing on the edge of their territories, and pairs approaching the shared boundary of a neighbouring territory where a

neighbouring pair was vocalizing. These behaviours may make vocal interactions between neighbouring animals more efficient, considering the limitations of sound transmission we found here, by reducing the distance between signalers and receivers. Another behaviour that may help to increase the transmission of the sounds is the use of higher perches for vocalizing, and the advantage of this behaviour was corroborated by our results. We found that male solo songs and duets were transmitted and received with less degradation (higher signal-to-noise ratio and lower excess attenuation) at higher perches, as has been observed in other species in a variety of different types of habitat (Dabelsteen et al., 1993; Krams, 2001; Mathevon et al., 2005; Barker et al., 2009).

Some acoustic signals evolved with acoustic characteristics that favour highest levels of degradation and attenuation, because the context of production may require privacy (e.g., mating signals) or help to prevent eavesdropping by competitors, predators, or parasites (Dabelsteen 2005). Probably, acoustic characteristics that favour the highest levels of degradation and attenuation observed in the solo songs and duets of White-eared Ground-sparrows are maintained because help to prevent that the signal may be eavesdropped by potential ambushed predators.

In White-eared Ground-sparrows, duets are vocalizations used mainly for communication within pairs (Chapter 2) and possibly with neighbouring pairs during interactions (Chapter 6). If the primary receiver for ground-sparrow duets is the bird's partner, located on the same territory, there may be little necessity for this vocalization to transmit long distances. This stands in contrast to the function of male solo songs, vocalizations used mainly for mate attraction (Chapter 2) and possibly territory defense (Chapter 6). If potential receivers are more than one territory width away, we would expect animals to produce vocalizations that transmit

over such distances, but this is not the case. However, field observations of two males that lost their partner during the breeding season suggest that males may change their vocal behaviour to enhance signal transmission. In the case of the two bachelor males, we observed birds singing from perches that varied from 8 to 15 m height; this is three to five times higher than average singing perches observed during the mornings in males with pairs ( $2.3 \pm 0.1$  m;  $N = 9$  males). A future transmission experiment using solo songs at these heights is encouraged to evaluate the possibility that males may further enhance the transmission range of their mate-attraction solos or improve the conditions for hearing a vocal response by using higher perches than we studied here.

Thick vegetation is expected to increase the tail-to-signal ratio of an animal vocalization through reverberation (Slabbekoorn et al., 2002; Bradbury & Vehrencamp, 2011). This may cause little distortion or amplification on unmodulated tonal sound (Nemeth et al., 2006; Slabbekoorn et al., 2002; Barker et al., 2009), but for the dramatic frequency-modulated sounds of ground-sparrows, the tail serves to distort the signal (Ryan & Brenowitz, 1985; Brumm & Naguib, 2009), although may contain information about the distance to the sender (Holland et al., 2001). Ground-sparrow solo songs and duets showed higher tails when the sounds were produced from higher perches and received closer to the ground. This effect that might be driven by stronger wind levels at these heights, as suggested in other studies (Barker et al., 2009), but likely arises due to the thick ground vegetation that characterized thicket habitats.

We also analyzed the degradation and attenuation in isolated elements of solo songs and duets because the differences in the frequency, duration, and modulation (Figure 4.1) are factors that play a primary role in how the sounds propagate throughout the environment (Wiley, 1991; Slabbekoorn et al., 2002; Ey & Fisher, 2009). The combination of the different

elements within solo songs and duets is expected to affect how the complete signal propagates throughout the environment (Slabbekoorn et al., 2002). Each element of the solo songs and duets showed similar degradation patterns to those observed for complete sounds. Excess attenuation, blur ratio, and tail-to-signal ratio increased with distance and proximity to the ground; meanwhile the signal-to-noise ratio decreased. Although we found differences between elements in degradation, we failed to find a significant difference between solo song and duet elements.

Degradation of solo song and duet characteristics may represent cues of the distance and position of the signalers (Morton, 1986; Naguib, 1995; Sabatini et al., 2011), given that sound degradation varied with both factors in White-eared Ground-sparrows. The evolution of vocalizations that provide information on the exact position of the signaler may enhance the efficiency of communication in closed habitats, like thickets where visual signals are limited even at close distances. This idea needs further investigation.

## **Conclusions**

Although many bird species in tropical habitats produce solo songs and duets (Langmore, 1998; Gil & Gahr, 2002; Hall, 2009), this is the first study to directly compare the transmission properties of solo song and duets in the same species. We found that both vocalizations showed the same pattern of degradation relative to the distance, supporting our prediction that both vocalizations are designed to communicate with receivers at similar distances when both sounds are emitted with the same level and the receivers are located at the same height above ground level. More comparative transmissions studies are necessary to understand the role of both vocalizations in the communication between signaler and potential receivers, especially for

species where duets are comprised of different type of vocalizations than vocalizations used for solo songs, as is the case for our study species here. For example if solo songs travel larger distances than duets with less degradation, it suggests the main function of this vocalization is likely to attract females that are far away; in contrast duets are likely used for close-range communication. It is important to analyze the transmission properties of calls because some of them may be used in close-range and long-range communication; there are very few transmission studies of calls to date.

Our results showed that solo songs and duets of White-eared Ground-sparrows degraded faster than observed in other species' vocalizations in other transmission studies; this pattern stands in contrast to the Acoustic Adaptation Hypothesis that predicts that sounds have characteristics that enhance the transmission inside the habitat where they are transmitted (Boncoraglio & Saino, 2007; Ey & Fisher, 2009). Yet when we considered the distances between signalers and receivers, for both solo songs and duets, the high levels of degradation appear not to be a problem for communication because the message arrives before complete degradation to the potential receiver. Therefore, it is necessary to take into account the distance between signalers and receivers in the interpretation of the results of transmission studies, because not all species will need signals that transmit over long distances (Dabelsteen, 2005). As we expected, solo songs and duets have less degradation when they were produced and received from exposed perches. This confirms that both vocalizations experience more degradation closer to the ground, due the reverberations and the dense vegetation.

## References

- Andersson, M. (1994). *Sexual selection*. — Princeton University Press, Princeton, NJ.
- Balsby, T.J.S., Dabelsteen, T. & Pedersen, S.B. (2003). Degradation of whitethroat vocalizations: implications for song flight and communication network activities. — *Behaviour* 140: 695-719.
- Barker, N.K. (2008). Bird song structure and transmission in the neotropics: trends, methods and future directions. — *Ornit. Neotrop.* 19: 175-199.
- Barker, N.K. & Mennill 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.
- Biamonte, E., Sandoval, L., Chacón, E. & Barrantes, G. (2011). Effect of urbanization on the avifauna in a tropical metropolitan area. — *Landscape Ecol.* 26: 183-194.
- Blumenrath, S. H. & Dabelsteen, T. (2004). Degradation of great tit (*Parus major*) song before and after foliation: implications for vocal communication in a deciduous forest. — *Behaviour* 141: 935-958.
- 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. (2011). *Principles of animal communication*. 2nd ed. — Sinauer, Sunderland, MA.
- Brown, C.H., Gomez, R. & Waser, P.M. (1995). Old world monkey vocalizations: adaptation to the local habitat? — *Anim. Behav.* 50: 945-961.
- Catchpole, C.K. & Slater, P.J.B. (2008). *Bird song biological themes and variations*. — Cambridge University Press, Cambridge.
- Dabelsteen, T. (2005). Public, private or anonymous? Facilitating and countering eavesdropping. — In: *Animal communication networks* (McGregor, P., H., ed.). Cambridge University Press, Cambridge. p. 38-62.
- Dabelsteen, T., Larsen, O.N. & Pedersen, S.B. (1993). Habitat-induced degradation of sound signals: quantifying the effects of communication sounds and bird location on blur ratio, excess attenuation, and signal-to-noise ratio in blackbird song. — *J. Acoust. Soc. Am.* 93: 2206-2220.
- Derryberry, E.P. (2009). Ecology shapes birdsong evolution: variation in habitat and morphology explains variation in white-crowned sparrow song. — *Am. Nat.* 174: 24-33.
- Dingle, C., Halfwerk, W. & Slabbekoorn, H. (2008). Habitat-dependent song divergence at subspecies level in the grey-breasted wood-wren. — *J. Evol. Biol.* 21: 1079-1089.
- Ey, E. & Fisher, J. 2009. The “acoustic adaptation hypothesis” a review of the evidence from birds, anurans and mammals. — *Bioacoustics* 19: 21–48.
- Forrest, T.G. (1994). From sender to receiver: propagation and environmental effects on acoustic signals. — *Am Zool.* 34: 644–654.

- Geissmann, T. (2002). Duet-splitting and evolution of gibbon songs. — *Biol. Rev.* 77: 57-76.
- Gil, D. & Gahr, M. (2002). The honesty of bird song: multiple constraints for multiple traits. — *Trends Ecol. Evol.* 17: 133-141.
- Hall, M.L. (2009). A review of vocal duetting in birds. — *Adv. Study Behav.* 40: 67-121.
- Harvey, C.A., Komar, O., Chazdon, R., Ferguson, B.G., Finegan, B., Griffith, D.M., Martínez-Ramos, M., Morales, H., Nigh, R., Soto-Pinto, L., van Breugel, M. & Wishnie, M. (2008). Integrating agricultural landscapes with biodiversity conservation in the Mesoamerican hotspot. — *Conserv. Biol.* 22: 8-15.
- Hansen, P. (1979). Vocal learning: its role in adapting sound structures to long-distance propagation and a hypothesis on its evolution. — *Anim. Behav.* 27: 1270-1271.
- Holland, J., Dabelsteen, T., Pedersen, S.B. & Larsen, O.N. (1998). Degradation of wren *Troglodytes troglodytes* song: implications for information transfer and ranging. — *J. Acoust. Soc. Am.* 103: 2154-2166.
- Krams, I. (2001). Perch selection by singing chaffinches: a better view of surroundings and the risk of predation. — *Behav. Ecol.* 12: 295-300.
- Lampe, H.M., Larsen, O.N., Pedersen, S.B. & Dabelsteen, T. (2007). Song degradation in the hole-nesting pied flycatcher *Ficedula hypoleuca*: implications for polyterritorial behaviour in contrasting habitat-types. — *Behaviour* 144: 1161-1178.
- Langmore, N. E. (1998). Functions of duets and solo songs of female birds. — *Trends Ecol. Evol.* 13: 136-140.
- Mathevon, N., Dabelsteen, T. & Blumenrath, S. H. (2005). Are high perches in the blackcap *Sylvia atricapilla* song or listening posts? A sound transmission study. — *J. Acoust. Soc. Am.* 117: 442-449.
- Marler, P. A. (2004). Bird calls: a cornucopia from communication. — In: *Nature's music: the science of bird song* (Marler, P. & Slabbekoorn, H., eds.). Elsevier Academic Press, San Diego. p. 132-177.
- Matrosova, V.A., Blumstein, D.T., Volodin, I.A. & Volodina, E.V. (2011). The potential to encode sex, age, and individual identity in the alarm calls of three species of Marmotinae. — *Naturwissenschaften* 98: 181-192.
- McGregor, P.K. & Krebs, J.R. (1984). Sound degradation as distance cue in great tit (*Parus major*). — *Behav. Ecol. Sociobiol.* 19: 57-63.
- Morton, E.S. (1975). Ecological sources of selection on avian sounds. — *Am. Nat.* 109: 17-34.
- Morton, E.S. (1986). Predictions from the ranging hypothesis for the evolution of long distance signals in birds. — *Behaviour* 99: 65-86.
- Naguib, M. (1995). Auditory distance assessment of singing conspecifics in Carolina wrens: the role of reverberation and frequency-dependent attenuation. — *Anim. Behav.* 50: 1297-1307.
- 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: 3263-3264.
- Pedersen, S.B. (1998). Preliminary operational manual for signal processor SigPro.— Centre of Sound Communication, Odense University, Odense.

- Perla, B. S. & Slobodchikoff, C. N. (2002). Habitat structure and alarm call dialects in Gunnison's prairie dog (*Cynomys gunnisoni*). — *Behav. Ecol.* 13: 844-850.
- Redondo, P., Barrantes, G. & Sandoval, L. (2013). Urban noise influences vocalization structure in the house wren *Troglodytes aedon*. — *Ibis* 155: 621-625.
- Ryan, M.J. & Brenowitz, E.A. (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. — *American. Nat.* 126: 87-100.
- Ryan, M. J., Cocroft, R.B. & Wilczynski, W. (1990). The role of environmental selection in intraspecific divergence of mate recognition signals in the cricket frog, *Acris crepitans*. — *Evolution* 44: 1869-1872.
- Sabatini, V., Ruiz-Miranda, C. R. & Dabelsteen, T. (2011). Degradation characteristics of golden lion tamarin *Leontopithecus rosalia* two-phrase long calls: implications for call detection and ranging in the evergreen forest. — *Bioacoustics* 20: 137-158.
- Sanchez-Azofeifa, G.A., Harriss, R.C. & Skole, D.L. (2001). Deforestation in Costa Rica: a quantitative analysis using remote sensing imagery. — *Biotropica* 33: 378-384.
- Sandoval, L. & Mennill, D.J. (2012). Breeding biology of white-eared ground-sparrow (*Melospiza leucotis*), with a description of a new nest type. — *Ornit. Neotrop.* 23: 225-234.
- Slabbekoorn, H. (2004). Singing in the wild: the ecology of birdsong. — In: *Nature's music: the science of bird song* (Marler, P. & Slabbekoorn, H., eds.). Elsevier Academic Press, San Diego. p. 178–205.
- Slabbekoorn, H. & Peet, M. (2003). Birds sing at higher pitch in urban noise. — *Nature* 424: 267.
- Slabbekoorn, H. & Smith, T. B. (2002). Habitat-dependent song divergence in the little greenbul: an analysis of environmental selection pressures on acoustical signals. — *Evolution* 56: 1848-1858.
- Slabbekoorn, H., Eilers, J. & Smith, T.B. (2002). Birdsong and sound transmission: the benefits of reverberations. — *Condor* 104: 564-573.
- Stiles, G. & Skutch, A. (1989). *Guide to the birds of Costa Rica*. — Cornell University Press, Ithaca, NY.
- Stutchbury, B.J. & Morton, E. S. (2008). Recent advances in the behavioral ecology of tropical birds: the 2005 Margaret Morse Nice lecture. — *Wilson J. Ornith.* 120: 26-37.
- Wiley, R.H. (1991). Association of song properties with habitats for territorial oscine birds of eastern North America. — *Am. Nat.* 138: 973-993.

## Tables

**Table 4.1.** Main effects and two-factor interactions in the general linear models comparing the complete solo songs versus complete duets for each attenuation and degradation measurement.

	Signal-to-noise Ratio			Tail-to-Signal Ratio		Blur Ratio		Excess Attenuation	
	df*	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Model	36	344.98	<0.001	2.77	<0.001	4.12	0.001	146.57	<0.001
Distance	3	480.44	<0.001	128	<0.001	13.72	<0.001	193.76	<0.001
Speaker height	1	4.22	0.04	0.06	0.80	2.09	0.15	0.01	0.91
Microphone height	1	48.78	<0.001	0.78	0.38	7.47	0.006	16.07	<0.001
Sound	4	6.82	<0.001	8.38	<0.001	0.36	0.83	0.45	0.77
Distance x speaker height	3	12.08	<0.001	2.68	0.05	2.96	0.03	20.96	<0.001
Distance x microphone height	3	10.13	<0.001	6.12	<0.001	8.07	<0.001	28.16	<0.001
Distance x sounds	12	0.42	0.95	3.62	<0.001	1.22	0.26	0.23	0.99
Speaker height x microphone height	1	12.72	<0.001	20.24	<0.001	1.33	0.25	52.43	<0.001
Speaker height x sounds	4	0.32	0.86	1.68	0.15	2.95	0.02	0.07	0.99
Microphone height x sounds	4	0.93	0.45	2.15	0.07	0.49	0.06	0.13	0.97

\*For signal-to-noise ration, tail-to signal ratio, and excess attenuation the degrees of freedom of the error are 665, and for blur ratio 682.

**Table 4.2.** Variation in the four degradation measurements (average  $\pm$  SE) according to distance analyzed in the transmission experiment of complete solo song and duets, and solo song and duet elements. The same letter connecting different distances inside each degradation measurement mean no differences in post hoc test.

Distance (m)	Signal-to-Noise	Tail-to-Signal	Blur Ratio	Excess
	Ratio (dB)	Ratio (dB)		Attenuation (dB)
Solo songs and duets				
4	33.95 $\pm$ 0.49 (a)	-44.97 $\pm$ 0.99 (a)	0.26 $\pm$ 0.01 (a)	11.94 $\pm$ 0.51 (a)
8	30.95 $\pm$ 0.66 (b)	-43.55 $\pm$ 0.92 (b)	0.26 $\pm$ 0.03 (a)	10.23 $\pm$ 0.44 (b)
16	21.67 $\pm$ 0.57 (c)	-33.17 $\pm$ 0.83 (c)	0.31 $\pm$ 0.01 (b)	17.97 $\pm$ 0.73 (c)
32	11.30 $\pm$ 0.63 (d)	-25.76 $\pm$ 0.71 (c)	0.39 $\pm$ 0.01 (c)	23.46 $\pm$ 0.61 (d)
Solo song and duet elements				
4	40.17 $\pm$ 0.37 (a)	-26.79 $\pm$ 0.41 (a)	0.20 $\pm$ 0.008 (a)	11.01 $\pm$ 0.38 (a)
8	37.32 $\pm$ 0.47 (b)	-24.33 $\pm$ 0.43 (b)	0.19 $\pm$ 0.006 (b)	9.58 $\pm$ 0.33 (b)
16	26.49 $\pm$ 0.46 (c)	-18.33 $\pm$ 0.46 (c)	0.24 $\pm$ 0.006 (c)	17.76 $\pm$ 0.51(c)
32	15.83 $\pm$ 0.45 (d)	-15.7 $\pm$ 0.42 (d)	0.28 $\pm$ 0.006(d)	23.98 $\pm$ 0.44 (d)

**Table 4.3.** Main effects and two-factor interactions in the general linear models comparing the solo song elements versus duet elements for each attenuation measurement.

	Signal-to-Noise Ratio			Tail-to-Signal Ratio		Blur Ratio		Excess Attenuation	
	df	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Model	67	495.03	<0.001	306.75	<0.001	108.61	<0.001	40.18	<0.001
Distance	3	1006.18	<0.001	248.38	<0.001	55.82	<0.001	457.14	<0.001
Speaker height	1	231.34	<0.001	0.66	0.42	8.62	0.3	1.98	0.16
Microphone height	1	334.62	<0.001	93.18	<0.001	234.21	<0.001	35.76	<0.001
Sound	9	13.81	<0.001	102.45	<0.001	7.31	<0.001	3.12	0.001
Distance x speaker height	3	27.77	<0.001	12.42	<0.001	1.46	0.22	38.14	<0.001
Distance x microphone height	27	21.49	<0.001	18.73	<0.001	16.37	<0.001	45.49	<0.001
Distance x sounds	12	0.42	0.99	2.38	<0.001	1.9	0.004	0.55	0.97
Speaker height x microphone height	1	20.32	<0.001	94.01	<0.001	92.95	<0.001	114.43	<0.001
Speaker height x sounds	9	0.46	0.9	0.67	0.74	1.11	0.36	0.1	0.99
Microphone height x sounds	9	0.76	0.65	0.7	0.71	2.31	0.01	0.39	0.94

\*For all model components, the degrees of freedom of the error are 1373.

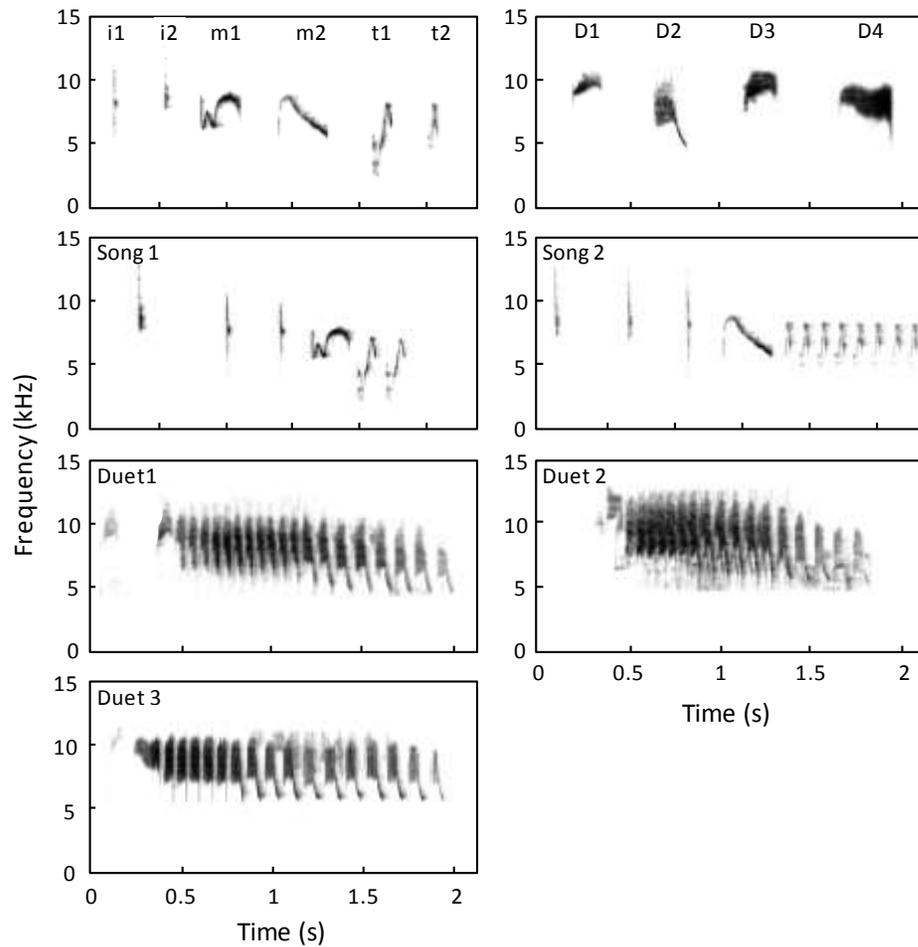
**Table 4.4.** Main effects and two-factor interactions in the two general linear models comparing the background noise across complete solo song and duets; and elements of solo songs and duets.

	Complete solo songs and duets			Elements solo songs and duets		
	df*	<i>F</i>	<i>p</i>	df <sup>†</sup>	<i>F</i>	<i>p</i>
Model	36	10.25	<0.001	66	10.39	<0.001
Distance	3	81.96	<0.001	3	155.02	<0.001
Speaker height	1	0.07	0.79	1	0.11	0.74
Micro height	1	0.0008	0.98	1	0.34	0.56
Element	4	0.03	0.99	9	0.1	0.99
Distance x speaker height	3	0.24	0.86	3	0.41	0.75
Distance x micro height	3	0.67	0.57	3	1.12	0.34
Distance x element	12	5.6	<0.001	27	4.91	<0.001
Speaker height x micro height	1	5.4	<0.001	1	12.03	<0.001
Speaker height x element	4	0.05	0.99	9	0.11	0.99
Micro height x element	4	0.21	0.93	9	0.14	0.99

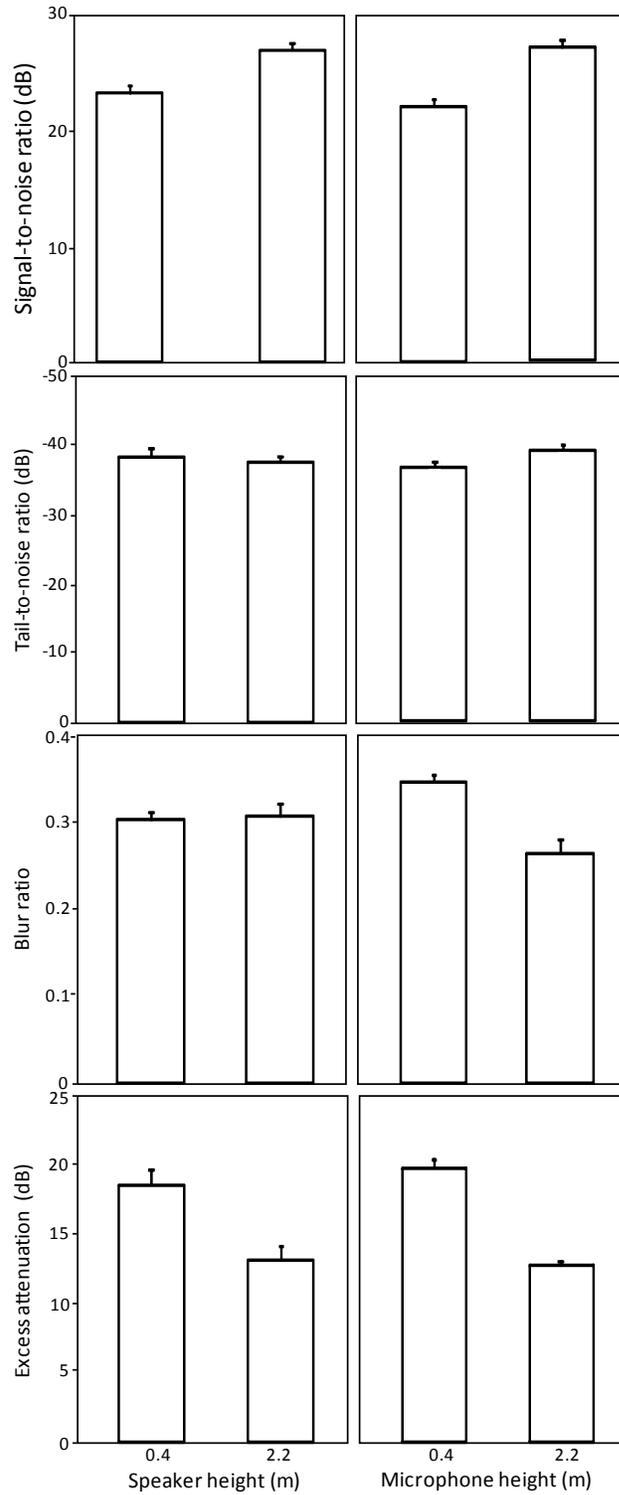
\*For all terms in complete solo songs and duets the degrees of freedom of the error are 203.

†For all terms in elements solo songs and duets the degrees of freedom of the error are 413.

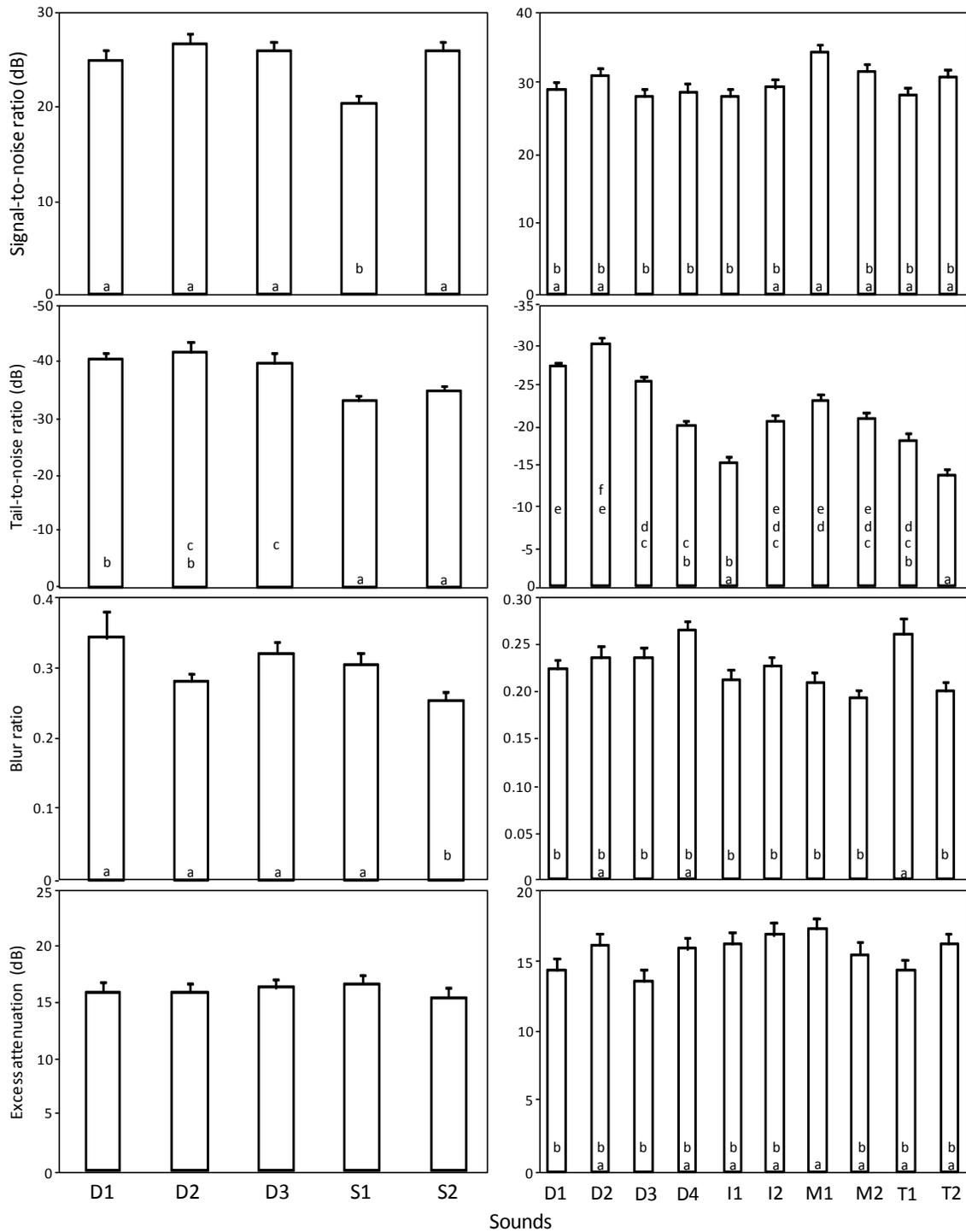
Figures



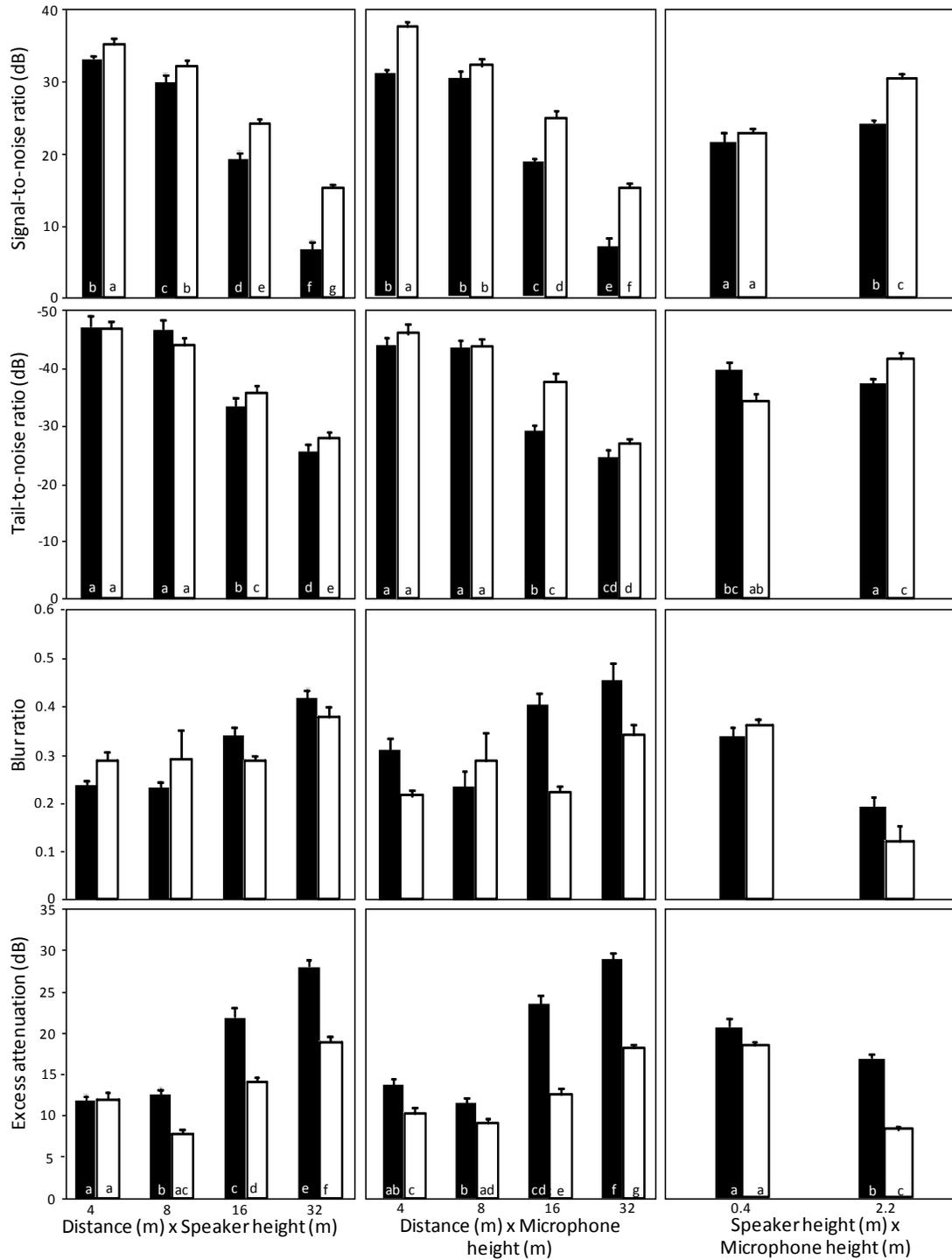
**Figure 4.1.** Spectrograms of the solo songs and duets, as well as the elements of solo songs and duets, of White-eared Ground-sparrows used in the transmission experiments. Letters indicate the code of each element and complete solo song (see Methods).



**Figure 4.2.** Variation in the four degradation measurements according to speaker and microphone heights used in the transmission experiments. Error bars are standard errors of the mean.



**Figure 4.3.** Variation in the four degradation measurements according to sounds for complete solo songs and duets, and solo song and duet elements used in the transmission experiments. Codes used in the sound column correspond with the letters in the figure 1. Error bars are standard errors of the mean. Bars with the same letters mean not differences in the post hoc test. Bars without letters mean no differences between each bar.



**Figure 4.4.** Second order interactions between distance and speaker and microphone heights (black, 0.4 m; white, 2.2 m) for complete solo songs and duets. Error bars are standard errors of the mean. Bars with the same letters mean no differences in the post hoc test. Bars without letters mean no differences between each bar.

## **Chapter 5**

### **Individual distinctiveness in the fine structural features and repertoire characteristics of the song of white-eared ground-sparrows\***

---

\*This chapter is the outcome of joint research with C. Méndez and D. Mennill

### **Chapter summary**

Communication between social animals is often more effective when signals facilitate individual recognition. Two critical requirements for individual recognition are the occurrence of characteristics that are unique to each individual, and the consistency of these characteristics through time. In some animals, characteristics of acoustic signals are known to vary over time due to changes in a variety of factors, including physiological and environmental features. Such temporal variation requires careful evaluation when exploring the individual distinctiveness of animal signals. In this study we evaluate individual distinctiveness in the songs of male white-eared ground-sparrows *Melospiza leucotis* and the persistence of distinctive characteristics over time. We collected focal recordings from populations of banded ground-sparrows during two consecutive breeding seasons, including multiple recording sessions within each breeding season. We evaluated individual distinctiveness in fine structural acoustic features of songs. We also extended our analysis to repertoire characteristics, focusing on whether the relative frequency of song type use may provide cues to individual identity. We found that each male white-eared ground-sparrow sang individually distinctive songs, although their fine structural features varied between recording sessions. We found the frequency with which males sang different song types was also individually distinctive, and this feature varied little between recording sessions. Receivers may be able to use these distinctive characteristics to differentiate individuals over extended time periods; this may be especially important for species that engage in long-term social interactions, such as tropical birds that defend territories against rival conspecific animals throughout the year.

## Introduction

A prerequisite for individual recognition is the occurrence of features that are unique to each individual, a characteristic that is common to many animals (Barnard & Burk 1979; Dale et al. 2001; Tibbetts & Dale 2007). In social groups, signals of identity allow receivers to distinguish between different signallers, making social interactions direct and efficient (Beecher 1982; Dale et al. 2001; Ellis 2008). For example, a receiver can judge if the signal comes from a competitor, a familiar neighbour, a breeding partner, a non-threatening juvenile, or a relative, and then respond according to the signaller's identity (van Rhijn & Vodegel 1980; Whitfield 1986; Tibbetts & Dale 2007; Wilson & Mennill 2010).

Individual-specific components of signals have been measured across a wide variety of animal species and multiple signalling modalities, including chemical, visual, and acoustic signals (Ptacek 2000; Ord & Stamps 2009; Tibbetts & Dale 2007; Grether et al. 2009; Grether 2011; Ord et al. 2011). Individually distinctive acoustic signals have been documented in amphibians (e.g. Bee et al. 2001; Feng et al. 2009; Gasser et al. 2009), mammals (e.g. Dallmann & Geissmann 2001; Blumstein & Munos 2005; Vannoni & McElligott 2007), and birds (e.g. Harris & Lemon 1972; Lovell & Lein 2004; Barrantes et al. 2008). Within birds, individually distinctive vocalizations have been reported in both song-learning species (e.g. Nelson & Poesel 2007; Ellis 2008; Benedict & McEntee 2009) and those with innate vocalizations (e.g. Lengagne et al. 2000; Fitzsimmons et al. 2008; Sandoval & Escalante 2011; Garcia et al. 2012). Individually distinctive vocalizations are thought to be more pronounced in song-learning species (Mennill 2011), especially because the learning process leads to small changes in acoustic structure, introducing "mistakes" and improvisations into the songs of each individual for song-learning species (Hultsch & Todt 2004; Catchpole & Slater 2008).

Another important component of individual recognition is the consistency of the individually distinctive features of signals through time. There is much evidence of signal consistency and of changes in signal characteristics over time, in many different avian taxa and other animal taxa (reviewed by Ellis 2008). In some bird species, fine structural characteristics of vocalizations may vary over time owing to morphological or physiological changes in the signaller (Nottebohm et al. 1987; Gil & Gahr 2002), or changes in the physical environment that serves as the transmission medium for the vocalizations (Forrest 1994, Slabbekoorn et al. 2002). Beyond fine structural features, broader characteristics of vocalizations may vary over time because of ontogenetic changes in repertoire size (e.g. Adret-Hausberger et al. 1990; Vargas-Castro et al. 2012) or changes in social status or breeding stage (e.g. Hennin et al. 2009, Topp & Mennill 2008). Consequently, it is important to measure temporal variation when evaluating the individuality of animal signals, especially in species that have more than one song type.

There are few studies that evaluate temporal variation in individually distinctive acoustic signals. Investigations of species with small repertoires have compared the fine structural acoustic characteristics of songs between recording sessions (e.g. Riebel & Salter 2003; Leitão et al. 2004; Ellis 2008; Wilson & Mennill 2010). Investigations of species with large repertoires have compared repertoire consistency through time (e.g. Adret-Hausberger et al. 1990; Todt & Hultsch 1998; Vargas-Castro et al. 2012). We were motivated by an interest in evaluating individual distinctiveness in a species with an intermediate to small repertoire size, to contrast two categories of vocal characteristics—fine structural features and repertoire characteristics—and gain a deeper understanding of individual distinctiveness in animal vocal signals.

In this study, we test whether male white-eared ground-sparrows *Melospiza leucotis* sing with individual distinctiveness, and whether this distinctiveness is found in the fine structural

acoustic features of their songs, or the broader characteristics of their repertoires, or both. Secondly, we analyze whether individually distinctive characteristics persist over time. White-eared ground-sparrows are year-round territorial songbirds that inhabit dense thickets, shade coffee plantations, and areas with early successional vegetation in Central America (Stiles & Skutch 1989; Sandoval & Mennill 2012). The visually-occluded nature of their habitat makes vocal signals the principal form of conspecific interaction for this species. Male white-eared ground-sparrows sing near their territory boundaries starting just before sunrise, and continue singing at a lower level throughout the course of the day; male solo songs have been associated with territory defence and mate attraction in this and other closely related species (Benedict & McEntee 2009; Sandoval & Mennill 2012; Chapter 6). If fine structural acoustic features or characteristics of their vocal repertoires are important for individual recognition, we expected that each male would exhibit unique fine structural features or repertoire characteristics. Furthermore if their acoustic features or repertoire characteristics are important in individual recognition we expected them to remain consistent over time.

## **Methods**

We recorded songs from 38 male white-eared ground-sparrows in four locations within Costa Rica (Figure 5.1): north of Heredia, Heredia province (10°01'N, 84°05'W; elevation: 1200-1500 m; n = 14 males); Universidad de Costa Rica campus, San José province (09°56'N, 84°05'W; elevation: 1200 m; n = 9); Lankester Botanical Garden, Cartago province (09°50'N, 83°53'W; elevation: 1400 m; n = 6); and Estación Biológica Monteverde, Puntarenas province (10°18'N, 84°48'W; elevation: 1600 m; n = 9). Although white-eared ground-sparrows produce solo songs,

duets, and calls, we focus on male solo songs here because these vocalizations are the most prominent for this species, and these vocalizations could be readily assigned to one individual.

We collected recordings between April and August 2011 and between March and June 2012, during this species' breeding season (Sandoval & Mennill 2012). Recordings were collected prior to egg laying, during incubation, and while the parents had hatchlings. For most of the birds sampled we were not able to calculate the exact stage of breeding because the dense thicket habitat at our study sites made finding nests and observing breeding behaviour difficult (e.g., in eight years of studying this species, we have found only ten nests; Sandoval & Mennill 2012). For 12 pairs in the current study, however, we observed the adults exhibiting nesting behaviour (adults carrying nesting materials or food) or we observed chicks directly, confirming that our recordings were collected during the breeding period.

We recorded each male between 0450 and 0600 h. We banded 35 of the 38 males with a unique combination of coloured leg bands. These individually-marked males allowed us to record the same individuals on different days during the same year and between years (the three unbanded males were recorded on a single day and are included only in our comparison of repertoire characteristics between males). We collected these recordings using a Marantz PMD660 or PMD661 digital recorder and a Sennheiser ME66/K6 shotgun microphone (recording format: WAVE; sampling rate: 44.1 kHz; accuracy: 16 bits). Twenty-one males were recorded during a single session in 2012; eleven males were recorded during two sessions in 2011 and one session in 2012; four males were recorded during three sessions in 2011 and one session in 2012; one male was recorded during two sessions in 2011; and one male was recorded during one session in each of 2011 and 2012. Each focal recording session lasted from 40 to 75 minutes (average  $\pm$  SE:  $59 \pm 1$  min). We complemented the repertoire size description for 10 males with

recordings obtained using autonomous digital recorders (model: Song Meter SM2; Wildlife Acoustics Inc., Concord, MA, USA) placed in the middle of the white-eared ground-sparrow territories. The location of these recorders in the middle of the birds' territories reduced the chance of our recording vocalizations from non-target individuals, particularly since ground-sparrow songs attenuate and degrade rapidly in this habitat, often in less than the width of one territory (Chapter 4). Each automated recording session lasted from 720 to 2160 minutes (average  $\pm$  SE:  $1368 \pm 168$  min).

#### Song Classification and Measurements

We classified song types visually based on their appearance on sound spectrograms (as in Franco & Slabbekoorn 2009, for example), focusing on the number of different types of elements and the overall shape of each element. All songs were compared to a library of song types that we developed for white-eared ground-sparrows. Within song types that share most of their features, we found subtle variation in the total number of elements; different birds added or omitted elements from the introductory component of the song, or varied the number of elements in the terminal trill. Songs that varied only in the number of repeats of introductory elements and terminal trill elements, but were otherwise similar in their fine structural details, we classified as the same song type (see Figure 5.2 for examples).

We measured two repertoire characteristics: the number of song types, and the frequency of use of each song type. We included in the comparisons only males with more than 20 songs recorded per male (average  $\pm$  SE:  $94.8 \pm 11.1$  songs per male,  $n = 38$  males), and between recording sessions of the same male with more than 12 songs within each session within and between years ( $54.8 \pm 7.1$  songs per session,  $n = 13$  males). We calculated male

repertoire size following the curve-fitting method with the Wildenthal equation (Wildenthal 1965). We conducted these repertoire size estimations for 19 males where we had recorded 80 or more recorded songs. We used Excel 2007 to implement the curve-fitting method, and we reported the estimated repertoire size from the asymptote of the curve.

For each song we measured seventeen fine structural acoustic features (Figure 5.3) using Raven Pro 1.4 sound analysis software (Cornell Lab of Ornithology, Ithaca, NY, USA). We measured duration, number of elements, lowest and highest frequency, and frequency of maximum amplitude for the entire song, the middle section of the song, and the terminal trill. In addition, we measured the number of inflections for elements in the middle portion of the song and the terminal trill (Figure 5.3). We used a combination of spectrograms (to identify the songs), power spectra (to measure frequency), and waveforms (to measure duration) to collect these 17 measurements. We used a temporal resolution of 5.8 ms and a frequency resolution of 188 Hz with the following settings: Hann window, 50% overlap, 256 kHz transform size. We collected these measurements in a subset of vocalizations from all of the vocalizations available from each male, selecting the first eight songs per song type per recording session for each male, skipping recordings that had prominent background sounds.

### Statistical Analysis

Different males often share the same song types between their repertoires, but they might sing these shared song types in different proportions, giving rise to a behaviour that may be individually distinctive. Hereafter, we refer to this behaviour of producing song types in similar proportions over time as “repertoire-use similarity”. To measure whether repertoire-use similarity can provide a cue of individual identity, we calculated the Morisita index of similarity

(Morisita 1959), to quantify the frequency of use of each song type, both between males and within males between recording sessions. We calculated the index according to the equation presented by Morista (1959; page 75); this index has values from 0 to 1, where values close to zero indicate 0% similarity between a pair of recordings, and values close to one indicate 100% similarity between a pair of recordings. For example, imagine three different birds that sing three song types (A, B, and C). If bird 1 sings type A 80% of the time, type B 20% of the time, and type C 0% of the time; bird 2 sings type A 60% of the time, type B 35% of the time, and type C 5% of the time; and bird 3 sings type A 20% of the time, type B 20% of the time, and type C 60% of the time; then bird 1 versus 2 has a Morisita similarity score of 0.94, bird 1 versus 3 has a Morisita similarity score of 0.36, and bird 2 versus 3 has Morisita similarity score of 0.48. We used cluster analysis to depict the pattern of repertoire-use similarity based on the Morisita scores. We evaluated repertoire-use similarity between populations and between recording sessions within males using one-way analysis of similarities (ANOSIM; Clarke 1993; Hammer 2012), where we used Morisita indices of similarity as the distance measurement.

We also conducted a Mantel test (using 10000 permutations) to evaluate the relationship between the geographic distances between the centre of males' territories (using Euclidian distance) and repertoire-use similarity scores (i.e. Morisita similarity scores). To analyze whether repertoire-use similarity between sessions is an effect of correlation in recording length, we ran an additional ANOSIM using Jaccard's index of similarity as the distance measurement. Jaccard's index of similarity compares only the repertoire size within males across recording sessions without taking into account the number of songs recorded in each session (as in Lapierre et al. 2011, for example). We used the following equation implemented in PAST (Hammer 2012):

$$J_i = \frac{M}{M + N}$$

where  $M$  is the number of songs shared by two males, and  $N$  is the total number of song sang just by one male.

Following the approach used by Ellis (2008), we used a discriminant function analysis to compare differences in the seventeen fine structural acoustic features between males. We used an interactive backward stepwise discriminant analysis to find the fewest possible acoustic features to explain the largest possible amount of variation between individuals. Using SYSTAT (version 11.00.01; SYSTAT Software, Chicago, IL, USA) we started with a model that included all 17 measurements; we excluded from the discriminant analysis the variable with the lowest F-to-remove value; after each exclusion we cross validated the model (see below for description of cross validation approach) and we continued excluding variables until we obtained a model with the fewest variables that still provided the same or higher percentage of correct assignments relative to the original model that included all acoustic features. This analysis was conducted for each song type that was shared by more than five males and that was sung eight or more times by each male; six song types satisfied these criteria. We report classification accuracy from the discriminant function analysis based on the leave-one-out approach to cross validation (Krebs 1999). We used a binomial test to compare if the classification accuracy determined by the discriminant function analysis is higher than the classification expected by chance. Chance expectations were calculated by dividing one by the number of males included in each particular DFA.

We complement the discriminant analysis approach by calculating the Potential for Individual Coding scores (PIC scores; Vignal et al. 2004; Robisson et al. 1993), on the six song types used in the discriminant function analysis mentioned above. This approach estimates the

coefficient of variation in the song characteristics between males ( $CV_b$ ) and within males ( $CV_w$ ). We estimated the PIC as the ratio between the two coefficients of variation ( $CV_b/CV_w$ ), where  $CV_w$  is the mean value of the  $CV_w$  of all individuals. When PIC scores are  $> 1.0$  the measured feature will have the potential for individual distinctiveness. We compare whether the variability in song measurements was different between  $CV_b$  and  $CV_w$  using analysis of variance. For this analysis, we pooled together all recording sessions for each male.

We used multiple analysis of variance (MANOVA) to explore whether fine structural features varied between recording sessions of the same song type. We focused on the fine structural features that were detected by the discriminant function analysis as being important for individual distinctiveness. In these analyses we used only males that sang the same song type in more than one recording session. For this analysis we nested recording session within male identity and used the fine structural measurements of songs as dependent variables. We only conducted this analysis for song types that were present in five or more males in two or more recording sessions; three song types satisfied these criteria. For each MANOVA, we present the details of the whole model as well as recording session nested within male (i.e. Recording session [Male]) and between males.

We used PAST (version 2.17; Øyvind Hammer, Natural History Museum, University of Oslo, Norway) for ANOSIM, Mantel tests, and cluster analyses. All other analyses were conducted in SYSTAT. Throughout, values are reported as means  $\pm$  SE. We considered our results significant at  $p = 0.05$ , except for the analyses that included multiple comparisons (see Results) when we reported significance based on exact Bonferroni corrections.

## Results

We collected recordings from 38 male white-eared ground-sparrows from four different populations in Costa Rica during two consecutive breeding seasons. For 19 males where we had 80 or more song recorded, the repertoires varied in size from two to eight song types, with an average repertoire size of  $3.5 \pm 0.3$  song types.

### Fine structure analysis

We found 32 unique song types in our recordings of the 38 recorded males, although the six most common song types dominated our recordings (2282 of 3627, or 62.9% of all recorded songs, were the six song types depicted in Figure 5.2). The fine structural acoustic features of the six most common male song types of white-eared ground-sparrows showed substantial between-male variation. Six discriminant analyses, one for each of the six most common song types, consistently assigned songs to the correct male at levels that exceeded chance expectations (Table 5.1). The lowest percentage of correctly-assigned songs during cross-validation for the six song types analyzed was 72%. This high level of distinctiveness was reached with a subset of acoustic features, varying from four to eight features, as shown in Table 5.1. These features varied among the six song types, although some of them (e.g. structural feature 14, the highest frequency of the terminal trill) were important in all six song types.

For the six most common song types, we found PIC scores greater than 1.0 (Tables S5.1-S5.6) for most of the fine structural features (88 to 100% of features in Tables S5.1-S5.6), indicating a high level of individual distinctiveness in male white-eared ground-sparrow songs. Following correction for multiple comparisons, three features showed the highest levels of

individual distinctiveness across the six song types, according to the PIC analysis: the duration of the middle section ( $p < 0.001$  for all comparisons), the lowest frequency of the middle section ( $p < 0.001$  for all comparisons), the highest frequency of the middle frequency ( $p < 0.01$  for all comparisons), and the lowest frequency of the trill ( $p < 0.001$  for all comparisons).

#### Repertoire-use similarity

Male white-eared ground-sparrows share song types between individuals and between populations. We found substantial variation between males in repertoire-use similarity (i.e. the relative proportion in which different song types are produced over time). Six males received a Morisita score for repertoire-use similarity  $\leq 0.50$ , indicating that their patterns of repertoire use were dissimilar from all other males. Eight males received a Morisita score for repertoire-use similarity of 0.51 to 0.75, indicating that their song repertoire use was moderately similar. Seventeen males received a Morisita score for repertoire-use similarity between 0.76 to 0.95 indicating that their repertoire use was moderately-to-highly similar. Eight males received a Morisita score for repertoire-use similarity higher than 0.95, indicating that repertoire use was highly similar (Figure 5.4). Interestingly, although all eight males with the highest similarity shared territory boundaries (i.e. two pairs of males in adjacent territories, and four males in a cluster from one population), not all males that shared territory boundaries showed this high degree in repertoire similarity. These differences in repertoire-use similarity indicate that the repetition patterns used by males may provide cues for distinguishing between individuals (Figure 5.4). Nearby males (males within each population) were more similar in their patterns of repertoire-use similarity when compared to males from other populations (ANOSIM:  $R = 0.77$ ,  $p$

< 0.001, Figure 5.4). This pattern was also true when we analyzed the relationship between geographic distance and repertoire-use similarity (Mantel test:  $R = 0.40$ ,  $p = 0.001$ ).

#### Consistency over time

We analyzed consistency in fine structural features of male songs for the three most common song types. The fine structural characteristics of song types varied both between males and between recording sessions within males for the three common song types that we analyzed, including song type 1 (whole model,  $F_{78,1163} = 128.91$ ,  $p < 0.001$ ; recording session [male],  $F_{48,1037} = 17.20$ ,  $p < 0.001$ ; males,  $F_{24,733} = 28.90$ ,  $p < 0.001$ ), song type 3 (whole model,  $F_{78,855} = 75.53$ ,  $p < 0.001$ ; recording session [male],  $F_{48,761} = 9.17$ ,  $p < 0.001$ ; males,  $F_{24,538} = 6.12$ ,  $p < 0.001$ ), and song type 18 (whole model,  $F_{156,1251} = 49.56$ ,  $p < 0.001$ ; recording event [male],  $F_{102,1215} = 2.40$ ,  $p < 0.001$ ; males,  $F_{48,1047} = 9.69$ ,  $p < 0.001$ ). In other words, for all three song types analyzed, we found significant variation in fine structural features between males and between sessions of the same male.

We compared repertoire-use similarity between recording sessions for 13 male white-eared ground-sparrows. Patterns of repertoire use were more similar within different recording sessions of the same male than between recording sessions of different males. This was true when we took into account the number of songs recorded (ANOSIM using Morisita scores:  $R = 0.83$ ,  $p < 0.001$ , Figure 5.5). The same pattern held true when we analyzed the number of song types detected independently of the number of songs recorded (ANOSIM using Jaccard indices:  $R = 0.55$ ,  $p < 0.001$ ).

## Discussion

Male white-eared ground-sparrows have individually distinctive songs and singing styles. Both the fine structural features of male songs as well as the proportion of time spent singing each song type vary more between individuals than within individuals. This distinctiveness is evident when we compared between multiple recording sessions of the same male, although there was also significant variation between recording sessions. We also found that males recorded in the same population share similar patterns of repertoire use in comparison to males from other populations.

Our results suggest that the solo song repertoire (song types and frequency of use) encode sufficient information to distinguish male white-eared ground-sparrow identity, at both the population level and the individual level. This pattern has also been reported in other bird species such as common blackbird (*Turdus merula*; Rasmussen & Dabelsteen 2002), white-crowned sparrow (*Zonotrichia leucophrys*; Nelson & Poesel 2007), and skylark (*Alauda arvensis*; Briefer et al. 2009). In these examples, one component of male songs or singing behaviour is understood to encode individuality and another component is thought to encode geographic or group affiliation. In our study of white-eared ground-sparrows, the group level might be encoded in the features that are shared between the males in the same population (e.g. repertoire-use similarity), and individual distinctiveness might be encoded in features that vary most between individuals (e.g. fine structural features).

We found that the fine structural features of male songs were individually distinctive in white-eared ground-sparrows. The structural features that contributed most strongly to individual distinctiveness in the discriminant analysis were frequency measurements of the songs and the number of elements and inflections within the trills (Table 5.1). Not all of the

structural features we measured encode sufficient information to distinguish males. For the six most widespread song types that we measured, only four to eight of the seventeen fine structural measurements were included in our backwards discriminant analysis, suggesting that a subset of fine structural features may be most useful for encoding identity. As in previous studies (e.g. Robisson et al. 1993; Tripp & Otter 2006; Garcia et al. 2012) a combination of frequency and temporal measures were the most individually distinctive components. We found significant differences in fine structural measurements between recording sessions of the same male, as has also been found in previous studies (see Ellis 2009). For example, black-capped chickadees exhibit significant variation between recording sessions in individually-distinctive song features, and their responses to playback reveal that they perceive playback songs from different recording sessions as the same male (Wilson and Mennill 2012). We expect white-eared ground-sparrows would behave in the same fashion, given the significant PIC scores across recording sessions and the significant effect of the singer's identity in our analyses; playback experiments will be required to confirm this expectation.

Repertoire characteristics (such as repertoire-use similarity, or repertoire size) might be inefficient for individual recognition (Kroodsma 1976; McGregor & Avery 1986; Botero et al. 2007), because they would require assessment over long periods. Indeed, if identity can be assessed from the fine structural features of a single song, this will necessarily be more efficient than assessing multiple songs. However, repertoire characteristics might provide additional information in individual discrimination that complements or enhances individual distinctiveness of fine structural features (Hartshorne 1956; Krebs 1977; Hultsch & Todt 1981; Searcy & Andersson 1986). Our results support the idea that patterns of repertoire use may enhance individual recognition within this ground-sparrow species, and that potential receivers (e.g., neighbours, other rival males, potential mates) might use these acoustic features to distinguish

between males. Playback experiments could help to test this idea by independently altering the fine structural features of playback songs and the simulated pattern of repertoire use.

Consistency of individual signals through time may be a common feature for species where individuals have long-term and stable social interactions with other individuals, particularly in species where individuals live in social groups (Jones et al. 1993; Riesch et al. 2006; Wright et al. 2008). White-eared ground-sparrows defend territories year-round (Sandoval & Mennill 2012), often occupying the same territory for several years (L. Sandoval, pers. obs.), so that neighbourhoods have stable long-term membership. White-eared ground-sparrows will benefit from individual recognition because they may defend territories against familiar rivals year after year, and it is beneficial to display less aggressive responses against stable neighbours as predicted by the dear enemy hypothesis (Fisher 1954).

In the tropics, early successional habitats may pose challenges for signal transmission because of high attenuation rates due the dense vegetation (McGregor & Krebs 1984; Wiley 1991; Slabbekoorn & Smith 2002; Dingle et al. 2008). In contrast to the predictions of the acoustic adaptation hypothesis, the songs of male white-eared ground-sparrows do not appear to be well adapted for long distance transmission through the dense vegetation of their native habitat. Male solo songs have broad bandwidth frequency modulations and consistently feature trills (Figure 5.1). These characteristics are more often associated with open habitats, rather than habitats with dense vegetation (Morton 1975; Wiley 1991); these features would be expected to show more substantial degradation and attenuation in dense vegetation compared to narrow bandwidth song elements or non-trilled songs (Blumstein & Turner 2005; Boncoraglio & Saino 2006). Evaluation of the transmission properties of male songs through the white-eared ground-sparrow's native thicket habitat, and whether the individually distinctive components

persist over long transmission distances (as in Christie et al. 2004), is important for assessing whether the individually distinctive components identified here can withstand attenuation and degradation.

In conclusion, our results demonstrate that both the fine structural acoustic features of ground-sparrow songs and their patterns of repertoire use encode individual distinctiveness. Playback will be required to determine whether white-eared ground-sparrows use these individually distinctive acoustic features in individual recognition. This study also reveals that the individually distinctive characteristics show little variation over time, as is predicted for species that are engaged in long-term social interactions including year-round territorial interactions, a common feature for many species of tropical birds. Using a Morisita index of similarity, we found that patterns of repertoire delivery by white-eared ground-sparrow males reveal individual identity and these patterns are consistent over time. This feature has rarely been investigated in birds, because individuals would require integration over long periods of time to assess the repertoire composition. Nevertheless, we encourage other investigators to look at higher-order cues of individual distinctiveness and their consistency over time.

## References

- Adret-Hausberger, M., Güttinger, H. R. & Merkel, F. W. 1990: Individual life history and song repertoire changes in a colony of starlings (*Sturnus vulgaris*). *Ethology* 84, 265–280.
- Barnard, C. J. & Burk, T. 1979: Dominance hierarchies and the evolution of “individual recognition”. *J. Theor. Biol.* 81, 65–73.
- Barrantes, G., Sánchez, C., Hilje, B. & Jaffé, R. 2008: Male song variation of Green Violet-ear (*Colibri thalassinus*) in the Talamanca mountain range, Costa Rica. *Wilson J. Ornithol.* 20, 519–524.
- Bee, M. A., Kozich, C. E., Blackwell, K. J. & Gerhardt, H. C. 2001: Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. *Ethology* 107, 65–84.
- Beecher, M. D. 1982: Signature systems and kin recognition. *Am. Zool.* 22, 477–490.
- Benedict, L., & McEntee, J. P. 2009: Context, structural variability and distinctiveness of California towhee (*Pipilo crissalis*) vocal duets. *Ethology* 115, 77–86.
- Blumstein, D. T. & Munos, O. 2005: Individual, age and sex-specific information is contained in yellow-bellied marmot alarm calls. *Anim. Behav.* 69, 353–361.
- Blumstein, D. T. & Turner, A. C. 2005: Can the acoustic adaptation hypothesis predict the structure of Australian birdsong? *Acta Ethol.* 8, 35–44.
- Boncoraglio, G. & Saino, N. 2006: Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Func. Ecol.* 21, 134–142.
- Botero, C. A., Riveros, J. M. & Vehrencamp, S. L. 2007: Relative threat and recognition ability in the response of tropical mockingbirds to song playback. *Anim. Behav.* 73, 661–669.
- Briefer, E., Aubin, T. & Rybak, F. 2009: Response to displaced neighbours in a territorial songbird with a large repertoire. *Naturwissenschaften* 96, 1067–1077.
- Catchpole, C. K. & Slater, P. J. B. 2008: Bird song biological themes and variation. Cambridge Univ. Press, Cambridge.
- Charif, R., Clark, C. & Frisrup, K. 2004. Raven 1.2 User’s Manual. Ithaca, NY: Cornell Laboratory of Ornithology.
- Clarke, K. R. 1993: Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* 18, 117–143.
- Christie, P. J., Mennill, D. J. & Ratcliffe, L. M. 2004: Chickadee song structure is individually distinctive over long broadcast distances. *Behaviour* 141, 101–124.
- 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. *Am. Nat.* 158, 75–86.
- Dallmann, R. & Geissmann, T. 2001: Different levels of variability in the female song of wild silvery gibbons (*Hylobates moloch*). *Behaviour* 138, 629–648.
- Dingle, C., Halfwerk, W. & Slabbekoorn, H. 2008: Habitat-dependent song divergence at subspecies level in the grey-breasted wood-wren. *J. Evol. Biol.* 21, 1079–1089.

- Ellis, J. M. 2008: Decay of apparent individual distinctiveness in the begging calls of adult female white-throated magpie-jays. *Condor* 110, 648–657.
- Feng, A. S., Riedet, T., Arch, V. S., Yu, Z., Xu, Z., Yu, X. & Shen, J. 2009: Diversity of the vocal signals of concave-eared torrent frogs (*Odorrana tormota*): evidence for individual signatures. *Ethology* 115, 1015–1028.
- Fisher, J. B. 1954: Evolution and bird sociality. In: *Evolution as a process* (Huxley, J., Hardy, A. C. & Ford, E. B., eds). Allen & Uwin, London, England, pp. 71–83.
- Fitzsimmons, L. P., Barker, N. K. & Mennill, D. J. 2008: Individual variation and lek-based vocal distinctiveness in songs of the Screaming Piha (*Lipaugus vociferans*), a suboscine songbird. *Auk* 125, 908–914.
- Forrest, T. G. 1994: From sender to receiver: propagation and environmental effects on acoustic signals. *Am. Zool.* 34, 644–654.
- Franco, P. & Slabbekoorn, H. 2009: Repertoire size and composition in great tits: a flexibility test using playbacks. *Anim. Behav.* 77, 261–269.
- Garcia, M., Charrier, I., Rendall, I. & Iwaniuk, A. N. 2012: Temporal and spectral analyses reveal individual variation in a non-vocal acoustic display: the drumming display of the ruffed grouse (*Bonasa umbellus*, L.). *Ethology* 118, 292–301.
- Gasser, H., Amézquita, A., & Hödl, W. 2009: Who is calling? Intraspecific call variation in the arrobatid frog *Allobates femoralis*. *Ethology* 115, 596–607.
- Gil, D. & Gahr, M. 2002: The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol. Evol.* 17, 133–141.
- Grether, G. F. 2011: The neuroecology of competitor recognition. *Integr. Comp. Biol.* 51, 807–818.
- Grether, G. F., Losin, N., Anderson, C. N. & Okamoto, K. 2009: The role of interspecific interference competition in character displacement and the evolution of competitor recognition. *Biol. Rev.* 84, 617–35.
- Hammer, Ø. 2012. PAST, version 2.17. Reference manual. Oslo, Norway: Natural History Museum, University of Oslo.
- Harris, M. A. & Lemon, R. E. 1972: Songs of song sparrows (*Melospiza melodia*): individual variation and dialects. *Canadian J. Zool.* 50, 301–309.
- Hartshorne, C. 1956: The monotony-threshold in singing birds. *Auk* 83, 176–192.
- Hennin, H. L., Barker, N. K., Bradley, D. W. & Mennill, D. J. 2009: Bachelor and paired male rufous-and-white wrens use different singing strategies. *Behav. Ecol. Sociobiol.* 64, 151–159.
- Hultsch, H. & Todt, D. 1981: Repertoire sharing and song-post distance in Nightingales (*Luscinia megarhynchos* B). *Behav. Ecol. Sociobiol.* 8, 183–188.
- Hultsch, H. & Todt, D. 2004: Learning to sing. In: *Nature's Music. The Science of Birdsong* (Marler, P. & Slabbekoorn, H., eds). Elsevier, San Diego, CA, pp. 80–107.

- Hyman, J., Hughes, M., Searcy, W. A. & Nowicki, S. 2004: Individual variation in the strength of territory defense in male song sparrows: correlates of age, territory tenure, and neighbour aggressiveness. *Behaviour* 141, 15–27.
- Jones, B. S., Harris, D. H. & Catchpole, C. K. 1993: The stability of the vocal signature in phee calls of the common marmoset, *Callithrix jacchus*. *Am. J. Primat.* 31, 67–75.
- Krebs, C. J. 1999: *Ecological methodology*. Addison Wesley Longman, California.
- Krebs, J. R. 1977: The significance of song repertoires: the Beau Geste hypothesis. *Anim. Behav.* 25, 475–478.
- Kroodsmas, D. E. 1976: Effect of large song repertoires on neighbor ‘recognition’ in male song sparrows. *Condor* 78, 97–99.
- Lapierre, J. M., Mennill, D. J. & MacDougall-Shackleton, E. A. 2011: Spatial and age-related variation in use of locally common song elements in dawn singing of song sparrows *Melospiza melodia*; old males sing the hits. *Behav. Ecol. Sociobiol.* 65, 2149–2160.
- Leitão, A., van Dooren, T. J. M. & Riebel, K. 2004: Temporal variation in chaffinch *Fringilla coelebs* song: interrelations between the trill and flourish. *J. Avian Biol.* 35, 199–203.
- Lengagne, T., Aubin, T., Jouventin, P. & Lauga, J. 2000: Perceptual salience of individually distinctive features in the calls of adult king penguins. *J. Acoust. Soc. Am.* 107, 508–516.
- Lovell, S. F. & Lein, M. R. 2004: Song variation in a population of alder flycatchers. *J. Field Ornithol.* 75, 146–151.
- McGregor, P. K. & Avery, M. I. 1986: The unsung songs of great tits (*Parus major*): learning neighbors’ songs for discrimination. *Behav. Ecol. Sociobiol.* 18, 311–316.
- McGregor, P. K. & Krebs, J. R. 1984: Sound degradation as distance cue in great tit (*Parus major*). *Behav. Ecol. Sociobiol.* 19, 57–63.
- Mennill, D. J. 2011: Individual distinctiveness in avian vocalizations and the spatial monitoring of behaviour. *Ibis* 153, 235–238.
- Mennill, D. J. & Vehrencamp, S. L. 2005: Sex differences in singing duetting behaviour of neotropical rufous-and-white wrens (*Thryothorus rufalbus*). *Auk* 122, 175–186.
- Mennill, D. J. & Vehrencamp, S. L. 2008: Context-dependent functions of avian duets revealed by microphone-array recordings and multispeaker playback. *Current Biol.* 18, 1314–1319.
- Morisita, M. 1959: Measuring of interspecific association and similarity between communities. *Mem. Faculty Sci. Kyushu Univ. Series E Biol.* 3, 65–80.
- Morton, E. S. 1975. Ecological sources of selection on avian sounds. *Am. Nat.* 109, 17–34.
- Nelson, D. A. & Poesel, A. 2007: Segregation of information in a complex acoustic signal: individual and dialect identity in white-crowned sparrow song. *Anim. Behav.* 74, 1073–1084.
- Nottebohm, F., Nottebohm, M. E., Crane, L. A. & Wingfield, J. C. 1987: Seasonal changes in gonadal hormone levels of adult male canaries and their relation to song. *Behav. Neural Biol.* 47, 197–211.
- Ord, J. T. & Stamps, J. A. 2009: Species identity cues in animal communication. *Am. Nat.* 174, 585–593.

- Ord, J. T., King, L. & Young, A. R. 2011: Contrasting theory with the empirical data of species recognition. *Evolution* 65, 2572–2591.
- Podos, J., Huber, S. K. & B. Taft. Bird song: the interface of evolution and mechanism. *Annu. Rev. Ecol. Evol. Syst.* 35, 55–87.
- Ptacek, M. B. 2000: The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behav. Proc.* 51, 111–134.
- Rasmussen, R. & Dabelsteen, T. 2002: Song repertoires and repertoire sharing in a local group of blackbirds. *Bioacoustics* 13, 63–76.
- Riebel, K. & Salter, P. J. B. 2003: Temporal variation in male chaffinch song depends on the Singer and the song type. *Behaviour* 140, 269–288.
- Riesch, R., Ford, J. K. & Thomsen, F. 2006: Stability and group specificity of stereotyped whistles in resident killer whales, *Orcinus orca*, off British Columbia. *Anim. Behav.* 71, 79–91.
- Robisson, P., Aubin, T. & Bremond, J-C. 1993: Individuality in the voice of the emperor penguin *Aptenodytes forsteri*: adaptation to a noisy environment. *Ethology* 94, 279–290.
- Sandoval, L. & Escalante, I. 2011: Song description and individual variation in males of the common pauraque (*Nyctidromus albicollis*). *Ornitol. Neotrop.* 22, 173–185.
- Sandoval, L. & Mennill, D. J. 2012: Breeding biology of white-eared ground-sparrows (*Melospiza leucotis*), with a description of a new nest type. *Ornitol. Neotrop.* 23, 225–234.
- Searcy, W. A. & Andersson, M. 1986: Sexual selection and evolution of song. *Annu. Rev. Ecol. Syst.* 17, 507–533.
- Slabbekoorn, H. & Smith, T. B. 2002: Habitat-dependent song divergence in the little greenbul: an analysis of environmental selection pressures on acoustical signals. *Evolution* 56, 1848–1858.
- Slabbekoorn, H., Eilers, J. & Smith, T. B. 2002: Birdsong and sound transmission: the benefits of reverberations. *Condor* 104, 564–573.
- Stiles, F. G. & Skutch, A. F. 1989: A guide to the birds of Costa Rica. Cornell Univ. Press, Ithaca.
- Tibbetts, E. A. & Dale, J. 2007: Individual recognition: it is good to be different. *Trends Ecol. Evol.* 22, 529–537.
- Todt, D. & Hultsch, H. 1998: How songbirds deal with large amounts of serial information: retrieval rules suggest a hierarchical song memory. *Biol. Cybern.* 79, 487–500.
- Topp, S. M. & Mennill, D. J. 2008: Seasonal variation in the duetting behaviour of rufous-and-white wrens (*Thryothorus rufalbus*). *Behav. Ecol. Sociobiol.* 62, 1107–1117.
- Tripp, T. M. & Otter, K. A. 2006: Vocal individuality as a potential long-term monitoring tool for western screech-owls, *Megascops kennicottii*. *Can. J. Zool.* 84, 744–753.
- van Rhijn, J. G. & Vodegel, R. 1980: Being honest about one's intentions: an evolutionary stable strategy for animal conflicts. *J. Theor. Biol.* 85, 623–641.
- Vannoni, E. & McElligott, A. G. 2007: Individual acoustic variation in fallow deer (*Dama dama*) common and harsh groans: a source-filter theory perspective. *Ethology* 113, 223–234.

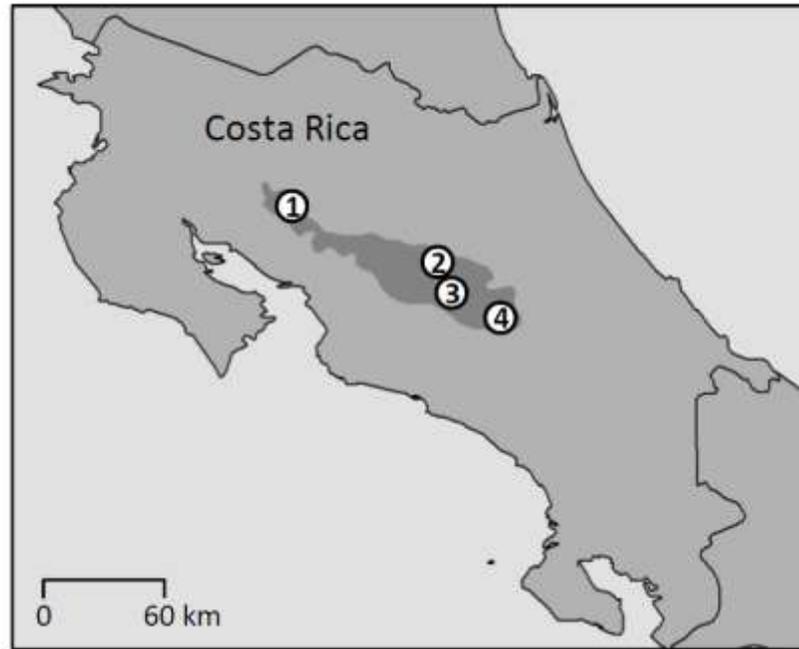
- Vargas-Castro, L. E., Sánchez, N. V. & Barrantes, G. 2012: Repertoire size and syllable sharing in the song of the clay-coloured thrush (*Turdus grayi*). *Wilson J. Ornithol.* 124, 446–453.
- Vignal, C., Mathevon, N. & Mottin, S. 2004: Audience drives male songbird response to partner's voice. *Nature* 430, 448–451.
- Wildenthal, J.L. 1965: Structure in primary song of the mockingbird (*Mimus polyglottos*). *Auk* 82, 161–189.
- Wiley, R. H. 1991: Association of song properties with habitats for territorial oscine birds of eastern North America. *Am. Nat.* 138, 973–993.
- Wilson, D.R. & Mennill, D. J. 2010: Black-capped chickadees (*Poecile atricapillus*) use individually distinctive songs to discriminate between conspecifics. *Anim. Behav.* 79, 1267–1275.
- Whitfield, D. P. 1986: Plumage variability and territoriality in breeding turnstone *Arenaria interpres*: status signalling or individual recognition? *Anim. Behav.* 34, 1471–1482.
- Wright, T. F., Dahlin, C. R. & Salinas-Melgoza, A. 2008: Stability and change in vocal dialects of the yellow-naped amazon. *Anim. Behav.* 76, 1017–1027.

## Tables

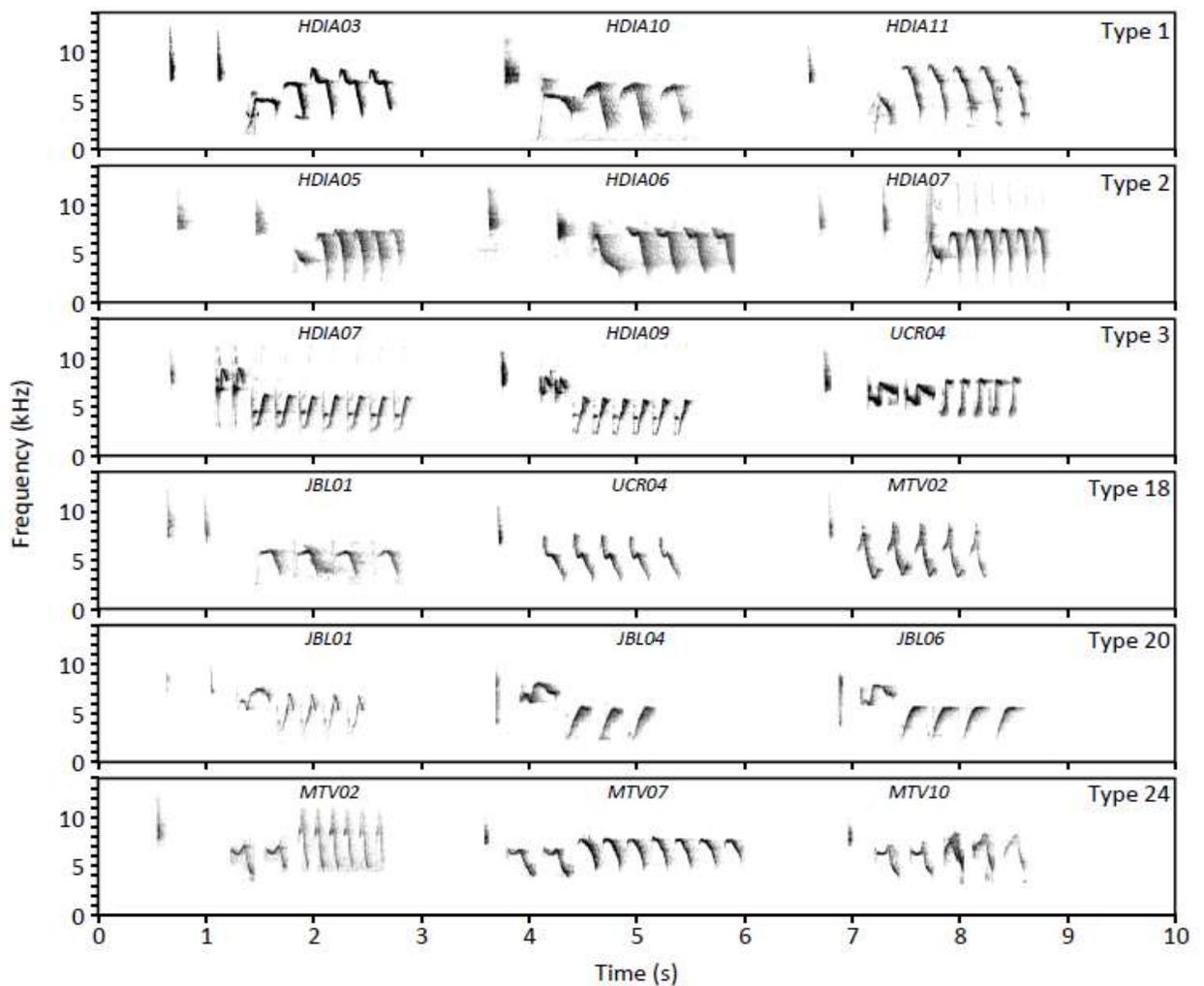
**Table 5.1.** Results of six discriminant function analyses (DFA) used to evaluate individual distinctiveness in male white-eared ground-sparrow songs. The analyses were conducted on six song types that were found in the repertoire of  $\geq 5$  males and that were sung  $\geq 8$  times for each male. Sample size (n) shows the total number of males that sang each song type in the analysis. The Wilks'  $\lambda$  and F values show the results of backwards DFA with cross-validation and  $p < 0.001$  for the six analyses. The p-values show the results of a binomial test comparing the percent of correct classification based on chance (i.e. one over n). The features retained in the backwards DFA correspond to the seventeen numbered fine structural features outlined in Figure 5.3.

Song Type	n	Percent Correct Classification	Wilks' $\lambda$	$F_{df}$	$p$	Features retained in backwards DFA
Type 1	10	88%	<0.001	36.6 <sub>72,634</sub>	<0.001	2, 8, 11, 12, 13, 14, 16, 17
Type 2	11	83%	<0.001	27.5 <sub>80,636</sub>	<0.001	2, 6, 7, 8, 11, 13, 14, 16
Type 3	10	80%	<0.001	28.8 <sub>72,731</sub>	<0.001	6, 7, 8, 12, 14, 15, 16, 17
Type 18	13	72%	<0.001	30.4 <sub>60,621</sub>	<0.001	2, 5, 14, 16, 17
Type 20	6	96%	<0.001	31.9 <sub>35,250</sub>	<0.001	4, 6, 7, 8, 12, 14, 16
Type 24	7	86%	<0.001	76.2 <sub>24,165</sub>	<0.001	2, 6, 8, 14

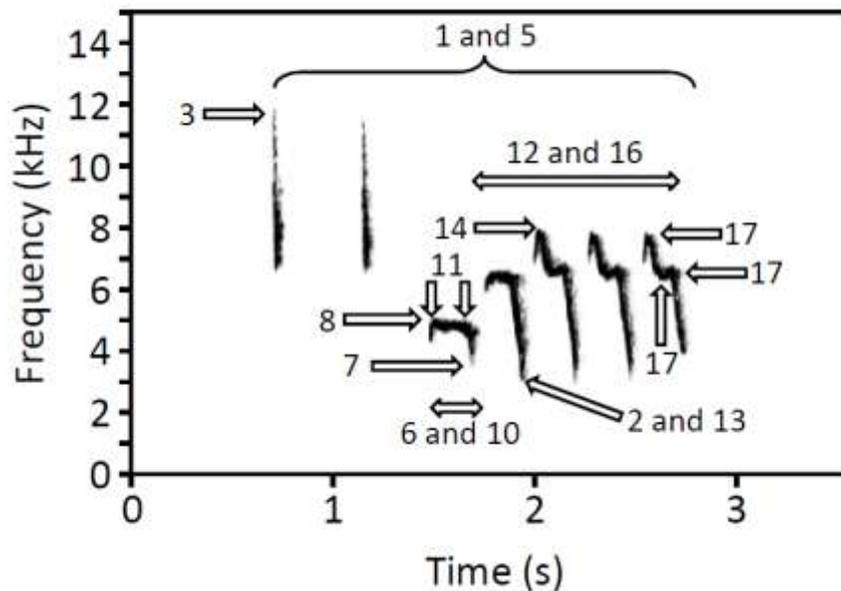
**Figures**



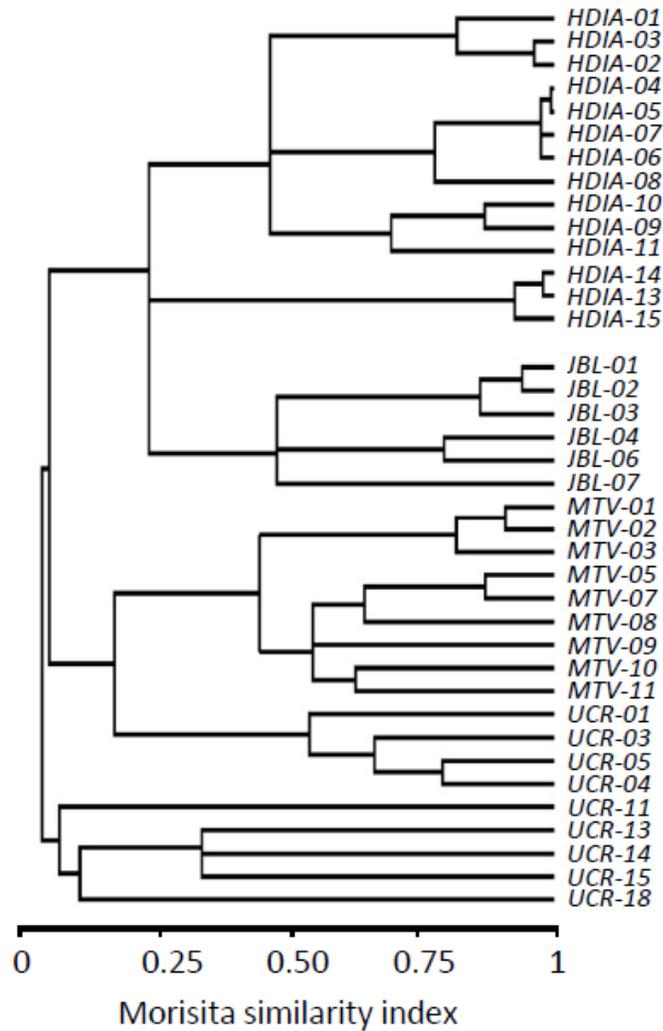
**Figure 5.1.** Map showing four populations of white-eared ground-sparrows in Costa Rica where male songs were recorded for analyses of individual distinctiveness: (1) Monteverde (MTV); (2) north of Heredia (HDIA); (3) the campus of Universidad de Costa Rica (UCR); and (4) Lankester Botanical Garden (JBL).



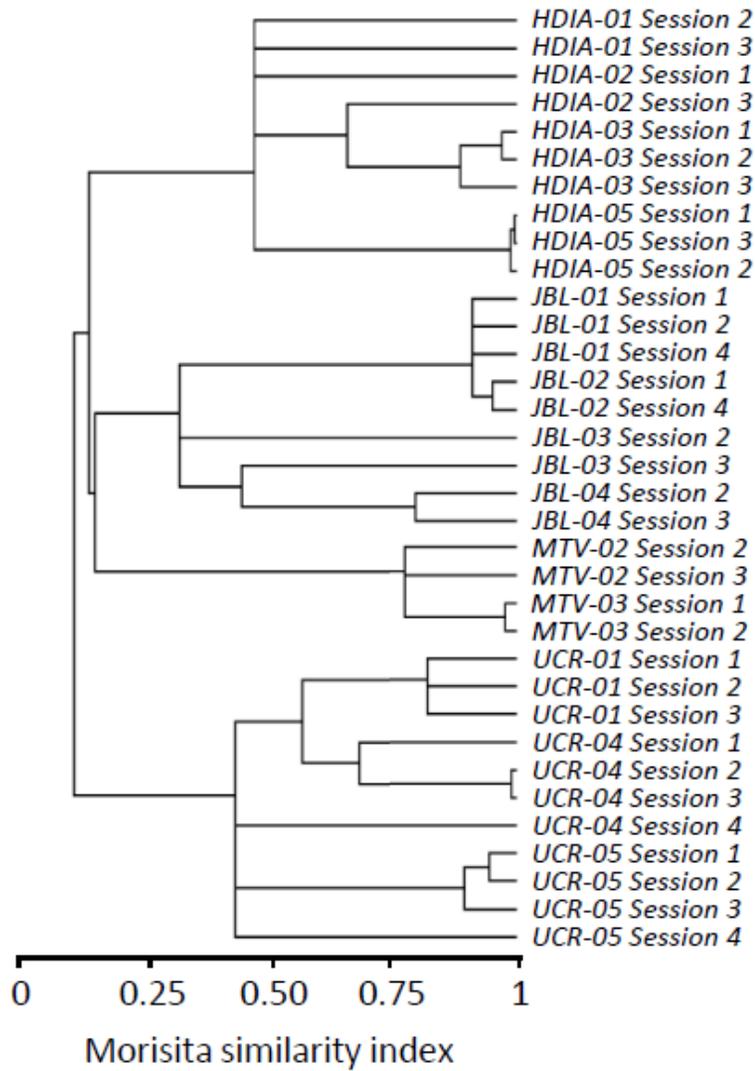
**Figure 5.2.** Sound spectrograms representing six common solo song types, with examples from three different male white-eared ground-sparrows for each type. Songs were classified visually according to similarities between the elements before the final trill, and overall song structure. Male identity is shown above each song, coded by the population of origin (HDIA: north of Heredia, JBL: Lankester Botanical Garden, MTV: Monteverde, and UCR: Universidad de Costa Rica campus) and a number to represent each individual.



**Figure 5.3.** Sound spectrogram of a typical white-eared ground-sparrow song, showing the 17 fine structural features we measured: (1) the duration of the whole song, in s; (2) the lowest frequency of the whole song, in Hz; (3) the highest frequency of the whole song, in Hz; (4) the frequency of maximum amplitude for the whole song (not shown); (5) the total number of elements of the whole song; (6) duration of middle section of the song (defined as the portion of the song following the high-pitched introductory notes and the start of the terminal trill), in s; (7) the lowest frequency of the middle section, in Hz; (8) the highest frequency of the middle section, in Hz; (9) the frequency of maximum amplitude for the middle section (not shown); (10) the total number of elements of the middle section; (11) the number of inflections points in the middle section; (12) the duration of the terminal trill, in s; (13) the lowest frequency of the terminal trill, in Hz; (14) the highest frequency of the terminal trill, in Hz; (15) the frequency of maximum amplitude for the terminal trill (not shown); (16) the total number of elements in the terminal trill; and (17) the number of inflection points in one syllable in the terminal trill.



**Figure 5.4.** Comparison between male solo song repertoire-use similarity in white-eared ground-sparrows, using the Morisita index of similarity, comparing shared song types and the frequency of utilization of each song type (N = 38). The tips of each branch show a letter code for the population where the bird was recorded (HDIA: north of Heredia, JBL: Lankester Botanical Garden, MTV: Monteverde, and UCR: Universidad de Costa Rica campus) and a number that represents the individual’s identity. When individuals are clustered at the end of branches, it means they show similar patterns of repertoire use.



**Figure 5.5.** Comparison between recording sessions of male solo song repertoire in white-eared ground-sparrows, using the Morisita index of similarity, comparing shared song types and the frequency of utilization of each song type within males ( $N = 13$ ). The tips of each branch show a letter code for the population where the bird was recorded (HDIA: north of Heredia, JBL: Lankester Botanical Garden, MTV: Monteverde, and UCR: Universidad de Costa Rica campus) and a number that represents the individual's identity.

## Chapter 6

**Different vocal signals, but not prior experience, influence heterospecific  
from conspecific discrimination\***

---

\*This chapter is the outcome of joint research with C. Mendéz and D. Mennill

### Chapter Summary

Efficient communication between animals requires specificity to ensure that animals distinguish relevant signals from background noise. Research on discrimination between the acoustic signals of heterospecific versus conspecific animals, especially in birds, has focused on the songs produced by breeding males, in spite of the fact that animals produce other types of acoustic signals such as calls and duets. We used acoustic playback experiments to evaluate whether tropical white-eared ground-sparrows, *Melospiza leucotis*, use calls, male solo songs and duets to discriminate conspecific from heterospecific competitors. We also evaluated whether prior experience influences competitors' discrimination by comparing responses among populations of white-eared ground-sparrows that are allopatric and sympatric with a congeneric competitor species (Prevost's ground-sparrows, *Melospiza biarcuata*). White-eared ground-sparrows displayed more intense responses to conspecific vocalizations than they did to congeneric vocalizations. The duets produced in response to conspecific playback exhibited higher bandwidth and maximum frequency, lower minimum frequency and longer duration than duets produced in response to heterospecific playback. These results suggest that white-eared ground-sparrows use information encoded in vocalizations to discriminate competitors from noncompetitor species. The observed responses were not influenced by previous experience; white-eared ground-sparrows displayed similar responses whether they lived in sympatry or allopatry with the congener simulated through playback. Our results expand our understanding of how animals use different types of vocalizations to discriminate conspecific from heterospecific signals.

## **Introduction**

Species specificity of animal signals is important for efficient communication (Bradbury & Vehrencamp 2011). Species-specific components of signals ensure that animals do not attend to signals that are not beneficial to their own interests, such as defending territories against heterospecifics that are not true competitors (Ryan & Rand 1993; Grether et al. 2009; Ord et al. 2011). The signals used by animals to distinguish their own species from potential competitors vary across taxa (Matyjasiak 2005; Bradbury & Vehrencamp 2011; Grether 2011), and are related to the modality of communication (Anderson & Gether 2010; Bradbury & Vehrencamp 2011) and also to individual discrimination (Marler 1960; Nelson 1989; Tibbetts 2002; Tibbetts & Dale 2004).

Acoustic signals have been particularly well studied as a species recognition signal, yet research on species discrimination via acoustic signals has focused almost exclusively on the songs produced by breeding males (Grether et al. 2009; Ord & Stamps 2009; Grether 2011; Ord et al. 2011). The primary functions of breeding males' acoustic signals are mate attraction and resource defence (Andersson 1994; Catchpole & Slater 2008). Species discrimination appears to be particularly important for male breeding signals because this reduces the chance of misidentification of relevant territorial competitors or prospective mates during the reproductive season (Murray 1981; Ptacek 2000; Ord & Stamps 2009; Grether 2011). Yet many animals, including birds, produce other types of acoustic signals beyond male breeding signals, such as calls and duets (Langmore 1998; Geissmann 2002; Marler 2004; Catchpole & Slater 2008; Furrer & Manser 2009; Bradbury & Vehrencamp 2011; Matrosova et al. 2011); these other types of signals may also include species-specific elements. Therefore, to understand the role of these others acoustic signals in conspecific and heterospecific discrimination, it is worthwhile to

conduct comparative studies between different categories of acoustic signals, rather than focusing on a single signal type.

Given the complexity and diversity of their vocalizations (Catchpole & Slater 2008), birds provide an excellent model for studying conspecific and heterospecific discrimination. The most biodiverse order of birds is Passeriformes, which is subdivided into oscine birds (suborder: Passeres) where birds learn songs from tutors, and suboscine birds (suborder: Tyranni) where birds inherit songs without learning (Kroodsma 2004). Although the mode of development of songs varies between these groups, calls appear to be nonlearned vocalizations for both groups (Marler 2004). Whether female songs and male–female duets are learned or innate is poorly understood; however, there are many species where duets comprise the same vocalizations as solo songs, suggesting that duets are probably learned in the same manner as male solo songs (e.g. Mennill & Rogers 2006). Based on the assumption that the songs and duets of oscine songbirds are learned, these vocalizations are more likely to show differences between species than are calls. These differences arise because songs and duets evolve under strong social evolutionary pressures (e.g. sexual preferences of the opposite sex for specific acoustic features, aggressive responses of same-sex animals to specific acoustic features, and the influence of neighbours vocalizations during periods of song learning) that are understood to lead to faster changes in culturally transmitted traits compared to genetically transmitted traits (Andersson 1994; Price 2007). Conversely, calls are more likely to show similarity between species than are songs or duets (e.g. Klump & Shalter 1984; Marler 2004; Templeton & Greene 2007). This similarity may arise because calls are used in interspecific communication, as is the case for mobbing calls, alarm calls or food calls (Marler 2004; Radford & Ridley 2007; Templeton & Greene 2007; Bradbury & Vehrencamp 2011).

In this study our objective was to conduct a comparative playback experiment to explore the role of different vocalization types (calls, male solo songs and male–female duets) for differentiating between heterospecific and conspecific competitors in Neotropical white-eared ground-sparrows, *Melospiza leucotis*. This songbird specializes in thicket habitats in Central America (Stiles & Skutch 1989; Howell & Webb 1995; Sandoval & Mennill 2012), which are characterized by dense vegetation where visual signals do not propagate well; vocal signals are therefore expected to be the dominant forms of communication and interaction between competitors in this habitat (Sandoval & Barrantes 2012). At different locations in Costa Rica, white-eared ground-sparrows live in sympatry or allopatry with respect to their closest relative, Prevost’s ground-sparrows, *Melospiza biarcuata* (Stiles & Skutch 1989; DaCosta et al. 2009). Duets and calls of these two ground-sparrows are superficially similar (Figure 6.1), so that there is ample opportunity for competitor misidentification when both species are present in the same area. As in *Hypocnemis* antbirds (Tobias & Seddon 2009; Seddon & Tobias 2010), and *Ficedula* flycatchers (Qvarnström et al. 2006), the vocal similarities between these two species, as well as their reliance on common resources, give rise to direct interactions between these two ground-sparrow species. Therefore, comparison of the sympatric and allopatric populations allowed us to evaluate the influence of vocal familiarity on the discrimination of heterospecific competitors.

We made a priori predictions about the responses of white-eared ground-sparrows pairs to playback simulating calls, solo songs, and duets of conspecific and congeneric animals. For responses to playback of male solo songs (which are known to be important in territory defence in this species, Sandoval & Mennill 2012, and in birds generally, Catchpole & Slater 2008), we predicted that both male and female white-eared ground-sparrows would show the highest intensity of response to conspecific signals versus congeneric signals (i.e. strong discrimination). We made this prediction for two reasons. First, the songs of the two congeners show substantial

spectrotemporal differences, more so than the other two types of vocalizations (Figure 6.1).

Second, males and females of the two congeneric species do not compete for breeding partners (there is no evidence of hybridization between white-eared and Prevost's ground-sparrows), so that white-eared ground-sparrows should show the highest intensity of response to conspecific signals. For responses to playback of vocal duets (which appear to be important in territory defence in white-eared ground-sparrows; Sandoval & Mennill 2012) and calls (which appear to serve as signals of alarm and contact signals in this species), we predicted that both male and female white-eared ground-sparrows would be less discriminating in their responses to conspecific versus congeneric competitors. We made this prediction for two reasons. First, unlike their songs, both the calls and the duets of these two species are very similar in fine structural features (Figure 6.1). Second, the cost of responding to the wrong species may be lower for duets and calls than for solo songs; given the function of these three types of signals (calls for alarm or contact, duets for territory defence, and solo songs for mate attraction), the cost of mistaking a congener for a conspecific should be highest for solo songs. If all vocalizations produced by white-eared ground-sparrows encode species information, we predicted a less aggressive response to all heterospecific vocalizations than to conspecific ones (Grether 2011). If species identity is not encoded in all vocalization types, we predicted the same intensity of response to conspecific and heterospecific vocalizations for those types of signals.

The ability to discriminate between acoustic signals may arise because the animals have an innate auditory template of the intraspecific signals or it may be learned (or modified) through experience (Ord et al. 2011). If discrimination is learned, then we predicted that birds living in sympatry would have frequent contact with the vocalizations of both congeneric and conspecific individuals during critical periods in their development, resulting in the ability to differentiate congeneric versus conspecific vocalizations (Catchpole 1978; Catchpole & Leister

1986). Conversely, birds living in allopatry would lack such experience, and should not show the ability to differentiate between the vocalizations that have high structural similarity (e.g. calls and duets). If species differentiation develops in the absence of learning through experience with the congener (i.e. if discrimination is a genetic trait), and the trait is shared across the allopatric and sympatric populations, then birds should respond more to conspecific than to congeneric vocalizations in both our allopatric and sympatric populations. It is possible that the genetic mechanism that facilitates conspecific from congeneric differentiation may have diverged between the sympatric and allopatric populations, in which case we predicted that birds living in sympatry would show the ability to differentiate congeneric versus conspecific vocalizations, whereas those living in allopatry would not.

## **Methods**

We studied four populations of white-eared ground-sparrows in Costa Rica from June to July 2011, during this species' breeding season (Sandoval & Mennill 2012). Two populations included white-eared ground-sparrows but no congeneric Prevost's ground sparrows: (1) Monteverde, Puntarenas Province (10°18'N, 84°48'W; altitude: 1600 m) and (2) Lankester Botanical Garden, Cartago Province (09°50'N, 83°53'W; altitude: 1400 m). We refer to these as 'allopatric populations' hereafter. The remaining two populations included coexisting white-eared ground-sparrows and Prevost's ground-sparrows: (3) North Heredia, Heredia Province (10°01'N, 84°05'W; elevation: 1200-1500 m) and (4) University of Costa Rica campus, San Jose Province (09°56'N, 84°05'W; elevation: 1200 m). We refer to these as 'sympatric populations' hereafter. In the sympatric populations, both species were found occupying the same type of habitat, and

they typically showed overlapping territories. We have observed the two species interacting with each other in the field.

#### Playback experiment

In all four study populations, we used playback to simulate the presence of four different species of birds inside the territories of white-eared ground-sparrows: (1) conspecific white-eared ground-sparrows; (2) congeneric Prevost's ground-sparrows; (3) a 'sympatric control', plain wrens, *Thryothorus modestus*; and (4) an 'allopatric control', large-footed finches, *Pezopetes capitalis*. We selected plain wrens as a sympatric control because they are common in the same habitat as white-eared ground-sparrows throughout their range in Costa Rica (Stiles & Skutch 1989), but they produce vocalizations that are highly different from ground-sparrows (Figure 6.1), and they are not known to be ecological competitors with ground-sparrows, feeding on different resources at different strata in the same habitat. We selected large-footed finches as an allopatric control because they live in similar habitats to both *Melospiza* species, but have a completely nonoverlapping distribution with white-eared ground-sparrows (Stiles & Skutch 1989), and therefore they are not ecological competitors. Large-footed finch vocalizations are somewhat similar in structure to white-eared ground-sparrow vocalizations, although they contain ample spectrotemporal differences (Figure 6.1). The two control species were also selected because they produce all three types of vocalizations (calls, solo songs and duets) of interest in our experiments (Figure 6.1).

We generated playback stimuli by isolating recorded vocalizations with a high signal-to-noise ratio (assessed visually) from recordings we collected in Costa Rica. Recordings were gathered with a shotgun microphone (Sennheiser ME66/K6) and a solid-state digital recorder

(Marantz PMD661; sampling rate: 44.1 kHz; accuracy: 16-bit; file format: WAVE). To minimize the effects of familiarity with vocalizations and any possible 'dear enemy effects' in our results (Temeles 1994; Catchpole & Slater 2008), we played back white-eared ground-sparrow vocalizations from the same geographical location but from the territory that was farthest from that of the playback subjects (minimum distance between the subject's territory and the stimulus bird's territory was two intervening territories). We used different stimuli for every pair. For the Prevost's ground-sparrow playback stimuli, we used vocalizations recorded from the two sympatric populations. For plain wren stimuli, we used recordings from the Central Valley. For large-footed finch stimuli, we used recordings from Cerro de la Muerte, Costa Rica.

We filtered out background noise outside of the range of the species' vocalizations using the Fast Fourier Transform filter function in Cool Edit 2000 (Syntrillium Software Co., Phoenix, AZ, U.S.A.). Each type of vocalization had different frequency characteristics, necessitating different types of filters. For white-eared and Prevost's ground-sparrows, we filtered all sounds below 3 kHz and all sounds above 12.5 kHz. For plain wrens, we filtered all sounds above 10 kHz; for calls and duets, we filtered all sounds below 2 kHz; and for songs, we filtered all sounds below 5 kHz. For large-footed finch, we filtered all sounds below 8 kHz and all sounds above 11 kHz for calls, all sounds below 2 kHz and above 6 kHz for songs, and all sounds below 1 kHz and above 10 kHz for duets (see Figure 6.1). The resulting filtered stimuli included only the signal of interest, allowing us to rule out the influence of background noise on the responses of the focal pair. We normalized all the recordings to -1 dB using the amplify function of Cool Edit 2000. After filtering and normalizing sounds, we confirmed that the filtered stimuli sounded realistic based on acoustic comparison to live birds in the field. All playback tracks consisted of one stimulus vocalization repeated several times. Each vocalization type differs in length; rather than holding playback rate constant, we held duty cycle constant. Calls were broadcast at a rate of 12

calls/min, songs were broadcast at a rate of eight songs/min, and duets were broadcast at a rate of four duets/min. These values also allowed us to broadcast stimuli at rates that corresponded with normal rates of delivery for these vocalizations based on our observations of wild birds, while still producing stimuli with an equivalent duty cycle between treatments.

Each playback trial included presentations of the same type of vocalization (calls, solo songs or duets) from each of the four species. Playback involved 2 min of vocalizations followed by 5 min of silence (Figure 6.2), with multiple trials in quick succession, similar to other experimental designs (e.g. Bolton 2007; Geberzahn et al. 2009; Ripmeester et al. 2010). We observed birds' response behaviour during playback and during the first 3 min of the silent period, and we treated the remaining 2 min of silence as a recovery period, allowing the focal pair to return to normal activities. Our field observations confirmed that birds consistently left the playback area by the end of the silent recovery periods. Within each trial we randomly selected the stimulus order (using the random number generator in Microsoft Excel), with the condition that we never presented vocalizations of two ground-sparrow species consecutively. Each focal pair received playback trials on 3 consecutive days (1 day receiving the four species' calls, 1 day receiving the four species' solo songs, 1 day receiving the four species' duets) where the order of trials followed a randomized design.

Playback of the four species were presented to 20 territorial white-eared ground-sparrows pairs in the allopatric populations (13 at Monteverde and 7 at Lankester Botanical Garden), and to 24 pairs in the sympatric populations (10 at Heredia and 14 at University of Costa Rica). Five pairs at each location had at least one individual banded, and our observations of these banded animals confirmed that they used the same territory during successive days and were not observed moving between territories throughout the breeding season. Therefore, we

are confident that the unbanded pairs that received playback were unique pairs. Playback sessions were conducted between 0600 and 1000 hours, a time when all four species were vocally active.

Playback sounds were broadcast using an active loudspeaker (Anchor Audio; Minivox; frequency response: 100–12000 Hz) and a portable audio player (Apple iPod classic). Loudspeakers were mounted at a height of 0.8–1.5 m, and were positioned inside the subjects' territory, 5–10 m from the edge of the territory. We hung flags at 3 m on either side of the loudspeaker to use as a reference during playback trials. Playback volume was held constant across all trials at 80 dB SPL, measured at 1 m from the speaker with a digital sound level meter (Radio Shack model 33-2055 using C weighting, slow response). We considered this to be similar to the amplitude of birds' voices based on our assessments in the field. Playback trials on different days were always broadcast from the same loudspeaker location, and the same observer was located at the same position, 8 m from the loudspeaker.

#### Response measures

We quantified birds' reactions to each playback stimulus by measuring both their behavioural responses (i.e. their physical reaction to playback) and the fine structural features of their vocal responses (i.e. their acoustic reaction to playback). We measured the following behavioural response variables: (1) the latency from the start of playback to the subjects' first vocalization, in seconds (if the pair did not vocalize we assigned a value of 300 s); (2) the latency to approach to within 3 m of the speaker, in seconds (if the pair did not approach we assigned a value of 300 s); (3) the time spent inside a 3 m radius from the speaker, in seconds (if the pair did not spend any time inside the 3 m radius we assigned a value of 0 s); and (4) the total number of

vocalizations produced during the 5 min, from the start of the playback to 3 min after playback finished.

Previous research shows that males may vary the structure of their vocalizations in response to playback experiments (e.g. Slabbekoorn & ten Cate 1997; Mennill & Ratcliffe 2004; Sandoval 2011; Bartsch et al. 2012). To evaluate whether ground-sparrows show similar behaviour, we measured the spectrotemporal characteristics of vocalizations produced by the focal pair during the 3 min of silence after each playback stimulus. Vocalizations produced during the 2 min of playback were often overlapped by playback and were therefore difficult to analyse in detail based on the sound spectrograms. The birds' vocalizations were recorded with a directional microphone (Sennheiser ME66/K6) and a solid-state digital recorder (Marantz PMD660 or PMD661). Using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.), for each recorded vocalization we measured: (1) the minimum frequency, in Hz; (2) the maximum frequency, in Hz; (3) the frequency bandwidth, in Hz; and (4) the duration, in seconds. We used Raven Pro 1.4 settings to achieve a temporal resolution of 5.8 ms and a frequency resolution of 188 Hz (settings: Hann window; 256 kHz transform size, and 50% overlap). The measurements were made through visual assessment of the spectrogram, wave and power spectrum windows in Raven Pro; the spectrogram window was used to identify the vocalization, and the wave and power spectrum windows were used to measure time and frequency limits, respectively. We calculated an average value when pairs produced more than one type of vocalization in response to playback.

### Statistical analysis

We conducted principal component analysis to combine the four behavioural responses into two multivariate response measures, using varimax rotation on the correlation matrix. The first two rotated components had eigenvalues greater than 1.0 and together explained 77.3% of the variance in the original four behavioural variables. The first rotated component explained 44.4% of the variation and showed a strong relationship with rapid approach to the loudspeaker ( $r = 0.93$ ; we present correlation coefficients between factor 1 and the raw variables) and time within 3 m of the loudspeaker ( $r = 0.93$ ), and a weak relationship with latency to first vocalization ( $r = 0.23$ ) and the total number of vocalizations produced ( $r = 0.04$ ). We call this first rotated component ‘close approach’, where pairs that received a high score approached rapidly and spent more time close to the speaker. The second rotated component explained 32.9% of the variation and showed a strong relationship with latency to first vocalization ( $r = 0.76$ ) and the total number of vocalizations produced in response to the stimulus ( $r = 0.85$ ), but a weak relationship with rapid approach to the loudspeaker ( $r = 0.14$ ) and time within 3 m of the loudspeaker ( $r = 0.60$ ). We therefore call this variable ‘song output’, where pairs that received a high score for this second principal component vocalized sooner and produced more vocalizations in response to the stimuli. The raw data for the behavioural measurements are presented in the Supplementary Material (Table S6.1).

We analysed variation in these two response variables using a linear mixed-effects model. We included the following four fixed factors: (1) the species that produced the stimulus (white-eared ground-sparrow, Prevost’s ground-sparrow, plain wren, large-footed finch); (2) the type of vocalization (call, solo song, duet); (3) whether the subjects lived in allopatry with Prevost’s ground-sparrows (allopatric or sympatric); and (4) the order of the playback stimulus

presentation (first, second, third or fourth stimulus of the day). We also included all second-order interactions between these four factors. To account for the fact that each pair was sampled repeatedly, we included subject identity as a random effect. The interaction between order of playback and species that produced the stimulus allowed us to evaluate whether responses varied with particular species being presented at particular positions within the stimulus set, and thereby assess position effects of playback order. We used the restricted maximum likelihood method for estimating fixed effects. For all factors or second-order interactions that explained significant variation in subjects' playback responses, we performed post hoc tests where we conducted all pairwise comparisons within each stimulus and vocalization type, followed by Bonferroni correction to account for multiple comparisons.

Focal pairs only produced calls and duets in response to playback; we never detected a solo song in response to playback. We analysed the structural features of subjects' calls and duets separately, because these vocalizations are structurally different (Figure 6.1) and presumed to be functionally distinct. We conducted principal component analysis to combine the four acoustic responses into one multivariate response measure for calls and one multivariate response measure for duets. For the analysis of calls, the first component had an eigenvalue greater than 1.0 and explained 54.6% of the variance in the original four variables. The first component showed a strong relationship with the frequency bandwidth ( $r = 0.97$ ), maximum frequency ( $r = 0.71$ ), minimum frequency ( $r = 0.63$ ) and duration ( $r = 0.51$ ). For the analysis of duets, the first component had an eigenvalue greater than 1.0 and explained 53.9% of the variance in the original four variables. The first component showed a strong relationship with the frequency bandwidth ( $r = 0.97$ ), minimum frequency ( $r = 0.76$ ), maximum frequency ( $r = 0.60$ ) and duration ( $r = 0.52$ ). Therefore, for both calls and duets, responses with a high principal component score had longer duration, broader bandwidth, higher maximum frequency and

lower minimum frequency. The raw data for the acoustics measurements are presented in the Supplementary Material (Tables S62, S63).

We conducted two linear mixed-effects model (one for calls, one for duets) to evaluate whether the characteristics of vocalizations produced in response to playback of the four species varied according to the type of vocalization and whether subjects lived in allopatry or sympatry with Prevost's ground-sparrows. We followed the exact same approach as in the first linear mixed-effects model for behavioural responses (above).

We used a significance threshold of  $\alpha = 0.025$  to reject the null hypothesis for these linear mixed-effects models, due to the fact that we conducted two comparisons of behavioural responses and vocal responses, instead of just one. All tests were two tailed. All values are reported as means  $\pm$  SE. All statistical analyses were conducted in JMP (version 10.0; SAS Institute, Cary, NC, U.S.A.) and SYSTAT (version 11.00.01; SYSTAT Software, Chicago, IL, U.S.A.).

#### Ethical note

In this study we presented acoustic stimuli that produced aggressive responses by territorial white-eared ground-sparrows. The aggression levels observed during playback trials were similar to the natural interactions we have observed between the focal species with other individuals of their own species and/or other species. We also observed the subjects for several minutes after conclusion of each experiment and confirmed that the focal pair resumed normal activities, similar to the behaviour they displayed prior to the experiment. We conducted this study following the regulations of the Animal Care Committee of the University of Windsor (AUPP: 09-06) and the Government of Costa Rica (071-2011-SINAC).

## Results

White-eared ground-sparrows responded strongly in many playback trials, often approaching the loudspeaker and producing calls and duets near the playback-simulated intruders. The approach responses of white-eared ground-sparrows, summarized by the first principal component (PC1), varied according to the species of intruder simulated through playback and the type of vocalization, as well as the interaction between these two factors (Figure 6.3a; linear mixed-effects model of variation in PC1; effect of playback species:  $F_{3,466} = 8.4$ ,  $P < 0.0001$ ; effect of playback vocalization type:  $F_{2,447} = 9.2$ ,  $P = 0.0001$ ; interaction of playback species and playback vocalization type:  $F_{6,447} = 6.6$ ,  $P < 0.0001$ ). Close approach responses did not vary between populations that were sympatric versus allopatric with respect to Prevost's ground-sparrows ( $F_{1,267} = 0.3$ ,  $P = 0.58$ ), nor did they vary with presentation order ( $F_{3,447} = 2.4$ ,  $P = 0.07$ ), or any of the remaining interaction terms (all  $F < 2.5$ ,  $P > 0.06$ ) including the interaction of presentation order and stimulus type ( $F_{9,473} = 0.9$ ,  $P = 0.55$ ). Post hoc analysis of the species simulated through playback revealed that white-eared ground-sparrows showed closer approach responses to all conspecific and congeneric vocalizations than to the two control species (Figure 6.3a). Post hoc analysis of stimulus type revealed that white-eared ground-sparrows showed a closer and faster approach to the duets than to songs and calls (Figure 6.4a). Post hoc analysis of the interaction between species and stimulus type revealed that white-eared ground-sparrows showed a closer and faster approach response to the duets of conspecific and congeneric playbacks than to solo songs and calls, whereas they showed no differences in response to the calls, solo songs and duets of the two control species (ANOVA:

white-eared ground-sparrow:  $F_{2,129} = 13.7$ ,  $P < 0.001$ ; Prevost's ground-sparrow:  $F_{2,129} = 7.0$ ,  $P = 0.001$ ; plain wren:  $F_{2,129} = 1.2$ ,  $P = 0.29$ ; large-footed finch:  $F_{2,129} = 1.9$ ,  $P = 0.16$ ; Figure 6.3a).

Song output, summarized by PC2, varied according to the species of intruder simulated through playback (linear mixed-effects model of variation in PC1, effect of playback species:  $F_{3,454} = 3.7$ ,  $P = 0.01$ ; Figure 6.3b). Song output did not vary between populations that were sympatric versus allopatric with respect to Prevost's ground-sparrows ( $F_{1,109} = 0.1$ ,  $P = 0.80$ ), type of vocalization ( $F_{2,447} = 3.0$ ,  $P = 0.05$ ) or presentation order ( $F_{3,447} = 1.5$ ,  $P = 0.21$ ), or any interaction terms (all  $F < 1.5$ ,  $P > 0.05$ ), including the interaction of presentation order and stimulus type ( $F_{9,458} = 1.2$ ,  $P = 0.29$ ). Post hoc analysis revealed that the species simulated through playback showed a significant effect; white-eared ground-sparrows showed higher song output in response to the conspecific and congeneric playbacks than they did in response to the two control species (Figure 6.3b).

In response to playback, white-eared ground-sparrows produced calls and duets, but never solo songs. Analysis of the fine structure of subjects' calls revealed that duration and frequency measurements did not differ significantly with simulated species ( $F_{3,347} = 0.5$ ,  $P = 0.72$ ; Figure 6.5a), vocalization type ( $F_{2,349} = 0.6$ ,  $P = 0.05$ ), sympatric versus allopatric population with respect to Prevost's ground-sparrows ( $F_{1,33} = 2.4$ ,  $P = 0.13$ ), presentation order ( $F_{3,349} = 0.5$ ,  $P = 0.72$ ) or any interaction terms (all  $F < 2.4$ ,  $P > 0.06$ ).

The fine structure of duets produced in response to playback varied according to the species of intruder simulated ( $F_{2,414} = 13.9$ ,  $P < 0.001$ ; Figure 6.5b) and the type of vocalization played ( $F_{2,414} = 16.9$ ,  $P < 0.001$ ; Figure 6.5c), but did not vary between populations that were sympatric versus allopatric with respect to Prevost's ground-sparrows ( $F_{1,39} = 5.3$ ,  $P = 0.026$ ), or with presentation order ( $F_{3,414} = 2.3$ ,  $P = 0.074$ ) or any interaction term ( $F < 1.5$ ,  $P > 0.18$ ). Based

on post hoc analysis, the species simulated through playback showed a significant effect; white-eared ground-sparrows produced duets with higher PC1 scores (i.e. higher maximum frequencies and bandwidths, lower minimum frequencies and longer durations) than to congeneric duets and duets of the two control species (Figure 6.5c).

### **Discussion**

Pairs of white-eared ground-sparrows displayed stronger responses to playback of conspecific calls, solo songs and duets compared to the same types of vocalizations from congeneric species and two unrelated control species. Duet playback incited the strongest responses in comparisons to calls and solo songs. Although subjects' responses varied according to the species and the type of vocalization simulated, responses were unrelated to previous experience; there were no differences in response to conspecific and congeneric playback between allopatric and sympatric populations.

Territorial pairs of white-eared ground-sparrows responded to playback of conspecific vocalizations by producing duets with longer duration, broader bandwidth, higher maximum frequency and lower minimum frequency (as summarized with a principal component score) in comparison to vocalizations they produced in response to the other three species. This result supports our prediction that white-eared ground-sparrow vocalizations encode species information, and that white-eared ground-sparrows distinguish conspecific from congeneric vocalizations. Therefore, the lack of difference in behavioural responses (approach behaviour and song output, explored below) towards Prevost's ground-sparrow duets and songs did not arise due to a lack of differentiation between their vocalizations. Instead, we think that the statistically similar behaviours shown towards the conspecific and congeneric playback arose

because the subjects recognized both species as ecological competitors (Grether 2011; Ord et al. 2011). Similar levels of aggressiveness are known, for example, in Virginia's warblers, *Oreothlypis virginiae*, and orange-crowned warblers, *Oreothlypis celata* (Martin & Martin 2001), and in collared flycatchers, *Ficedula albicollia*, and pied flycatchers, *Ficedula hypoleuca* (Qvarnström et al. 2006). In both of these examples, territorial birds responded similarly to signals of congenetics and conspecifics.

The behavioural responses of white-eared ground sparrows to calls of the four simulated species did not differ significantly. The similar behavioural responses to calls may arise due to similarity in call function between species (i.e. to communicate alarm or as a contact signal). We cannot distinguish whether birds failed to distinguish which species was simulated by call playback, or whether the birds recognized the species but responded in similar fashion to calls of the four species (Klump & Shalter 1984; Radford & Ridley 2007; Templeton & Greene 2007; Sandoval & Wilson 2012). Our results contrast with those of previous studies showing stronger responses to conspecific calls than to other species' calls, as in satin bowerbirds, *Ptilonorhynchus violaceus*, where males show stronger responses to calls from their own population (Nicholls 2008). The calls of satin bowerbirds are much more complex than the simple calls of the four species that we simulated in the current experiment, which may account for the differences between these studies.

White-eared ground-sparrows did not respond differently to congeneric Prevost's ground-sparrows vocalizations whether they were in zones of sympatry or allopatry. Birds living in two of our study populations have historically lived in isolation of this congeneric species (Slud 1964; Stiles & Skutch 1989), and yet they still discriminated between the two species based on playback. This supports our prediction that the mechanism for conspecific discrimination is

genetic and that the competitor recognition system has clearly not diverged between sympatric and allopatric populations; otherwise, we would have seen different responses in the sympatric versus allopatric populations. Previous investigations of two subspecies of *Sylvia* warblers (Brambilla et al. 2008) and populations of medium ground-finch (Podos 2007) showed that previous experience was not necessary to distinguish between competitors. For example, the two populations of medium ground-finch were separated by 11 km; males in each population responded more strongly to their own population's songs, even though the songs were not distinguishable by acoustic measurements (Podos 2007). In the case of *Sylvia* warblers, males of two subspecies show the same degree of reduced aggressiveness to the other subspecies song in allopatric and sympatric populations (Brambilla et al. 2008).

It is easy to imagine that white-eared ground-sparrows combine vocal signals (e.g. duets) with visual signals (e.g. plumage features) to distinguish conspecific from heterospecific competitors, as occurs in *Sylvia* warblers (Matyjasiak 2005). Our observations of birds' behaviour during playback support this idea; pairs rapidly approached playback of duets of both *Melospiza* species and they typically moved around the speaker, as if to search for the source of the sound (behaviours that were not observed during responses to the two control species). This behaviour is consistent with the idea that birds may have been searching for additional information, possibly in the form of plumage-based signals of species identity, although confirming this idea would require a complex experiment on the interplay of acoustic and visual signals in species discrimination.

By focusing on the responses of white-eared ground-sparrows to playback of their own species' calls, solo songs and duets, we can gain insight into the functions of these different signals. Interestingly, we found that territorial pairs showed their closest approaches and highest

song output in response to duets, and less intense responses to playback of solos and calls. If we interpret close approach and high vocal output as aggressive behaviours, these intense responses to duets compared to solo songs and calls offer strong support for the territory resource hypothesis for duet function in white-eared ground sparrows (Hall 2004). A similar pattern has been revealed previously in at least three other species of territorial duetting birds, although there are also duetting animals that respond with similar high intensity to solo songs and duets (reviewed in Hall 2009).

In conclusion, results of this playback study demonstrate that three different types of avian vocalizations may encode species information that facilitates discrimination between conspecific and congeneric competitors versus heterospecific noncompetitors (i.e. allopatric and sympatric controls). However, each type of vocalization elicits different intensities of response against conspecific and heterospecific rivals. To develop a better understanding of acoustic signals and their role in species discrimination (e.g. species recognition, competitor discrimination and mate selection), it is worthwhile to conduct comparative studies between all types of acoustic signals and avoid focusing on a single type of signal (e.g. solo songs). Our experiments using allopatric and sympatric population comparisons allow us to conclude that familiarity based on previous experiences and interactions between sympatric species are not a prerequisite for species-specific signal recognition, and our results suggest that this discrimination may be an innate process independent of experience with other species.

## References

- Anderson, C. N. & Grether, G. F. 2010. Character displacement in the fighting colours of *Hetaerina* damselflies. *Proceedings of the Royal Society B*, 277, 3669–3675.
- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Bartsch, C., Weiss, M. & Kipper, S. 2012. The return of the intruder: immediate and later effects of different approach distances in a territorial songbird. *Ethology*, 118, 876–884.
- Bradbury, J. W. & Vehrencamp, S. L. 2011. *Principles of Animal Communication*. 2nd edn. Sunderland, Massachusetts: Sinauer.
- Brambilla, M., Jannin, O., Guidali, F. & Sorace, A. 2008. Song perception among incipient species as a mechanism for reproductive isolation. *Journal of Evolutionary Biology*, 21, 651–657.
- Bolton, M. 2007. Playback experiments indicate absence of vocal recognition among temporally and geographically separated populations of Madeiran storm-petrels *Oceanodroma castro*. *Ibis*, 149, 255–263.
- Catchpole, C. K. 1978. Interspecific territorialism and competition in *Acrocephalus* warblers as revealed by playback experiments in areas of sympatry and allopatry. *Animal Behaviour*, 26, 1072–80.
- Catchpole, C. K. & Leister, B. 1986. Interspecific territorialism in reed warblers: a local effect revealed by playback experiments. *Animal Behaviour*, 34, 299–330.
- Catchpole, C. K. & Slater, P. J. B. 2008. *Bird Song Biological Themes and Variation*. Cambridge: Cambridge University Press.
- DaCosta, J. M., Spellman, G. M., Escalante, P. & Klicka, J. 2009. A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *Journal of Avian Biology*, 40, 206–216.
- Furrer, R. D. & Manser, M. B. 2009. The evolution of urgency-based and functional referential alarm calls in ground-dwelling species. *American Naturalist*, 173, 400–410.
- Geberzahn, N., Goymann, W., Muck, C. & ten Cate, C. 2009. Females alter their song when challenged in a sex-role reversed bird species. *Behavioral Ecology and Sociobiology*, 64, 193–204.
- Geissmann, T. 2002. Duet-splitting and evolution of gibbon songs. *Biological Reviews*, 77, 57–76.
- Grether, G. F. 2011. The neuroecology of competitor recognition. *Integrative and Comparative Biology*, 51, 807–818.
- Grether, G. F., Losin, N., Anderson, C. N. & Okamoto, K. 2009. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. *Biological Reviews*, 84, 617–635.
- Hall, M. S. 2004. A review of hypotheses for the functions of avian duetting. *Behavioral Ecology and Sociobiology*, 55, 415–430.
- Hall, M. S. 2009. A review of vocal duetting in birds. *Advances in the Study of Behavior*, 40, 67–121.

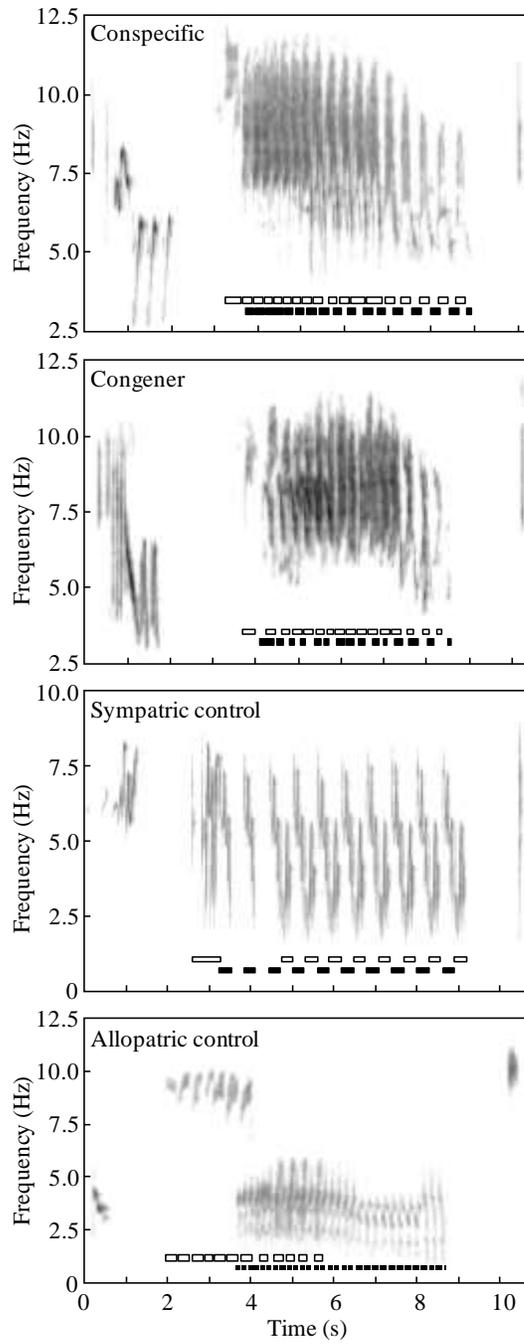
- Howell, S. N. G. & Webb, S. 1995. *A Guide to the Birds of Mexico and Northern Central America*. New York: Oxford University Press.
- Klump, G. M. & Shalter, M. D. 1984. Acoustic behaviour of birds and mammals in the predator context. I. Factors affecting the structure of alarm signals. II. The functional significance and evolution of alarm signals. *Zeitschrift für Tierpsychologie*, 66, 189–226.
- Kroodsma, D. 2004. The diversity and plasticity of birdsong. In: *Nature's Music: the Science of Bird Song* (Ed. by P. Marler & H. Slabbekoorn), pp. 108–131. San Diego: Elsevier Academic Press.
- Langmore, N. E. 1998. Functions of duets and solo songs of female birds. *Trends in Ecology & Evolution*, 13, 136–140.
- Marler, P. A. 1960. Bird songs and mate selection. In: *Animal Sounds and Communication* (Ed. by W. E. Lanyon & W. N. Tavolga), pp. 348–367. Washington, D.C.: American Institute of Biological Sciences.
- Marler, P. A. 2004. Bird calls: a cornucopia from communication. In: *Nature's Music: the Science of Bird Song* (Ed. by P. Marler & H. Slabbekoorn), pp. 132–177. San Diego: Elsevier Academic Press.
- Martin, P. R. & Martin, T. E. 2001. Behavioral interactions between coexisting species: song playback experiments with wood warblers. *Ecology*, 82, 207–218.
- Matrosova, V. A., Blumstein, D. T., Volodin, I. A. & Volodina, E. V. 2001. The potential to encode sex, age, and individual identity in the alarm calls of three species of Marmotinae. *Naturwissenschaften*, 98, 181–192.
- Matyjasiak, P. 2005. Birds associate species-specific acoustic and visual cues: recognition of heterospecific rivals by blackcaps. *Behavioral Ecology*, 16, 467–471.
- Mennill, D. J. & Ratcliffe, L. M. 2004. Overlapping and matching in the song contests of black-capped chickadees. *Animal Behaviour*, 67, 441–450.
- Mennill, D. J. & Rogers, A. C. 2006. Whip it good! Geographic consistency in male songs and variability in female songs of the duetting eastern whipbird (*Psophodes olivaceus*). *Journal of Avian Biology*, 37, 93–100.
- Murray, B. G., Jr. 1981. The origins of adaptive interspecific territorialism. *Biological Reviews*, 56, 1–22.
- Nelson, D. A. 1989. The importance of invariant and distinctive features in species recognition of bird song. *Condor*, 91, 120–130.
- Nicholls, J. A. 2008. Site specificity in advertisement calls and response to playbacks of local and foreign call variants in satin bowerbirds, *Ptilonorhynchus violaceus*. *Behavioral Ecology and Sociobiology*, 62, 831–841.
- Ord, J. T. & Stamps, J. A. 2009. Species identity cues in animal communication. *American Naturalist*, 174, 585–593.
- Ord, J. T., King, L. & Young, A. R. 2011. Contrasting theory with the empirical data of species recognition. *Evolution*, 65, 2572–2591.

- Podos, J. 2007. Discrimination of geographical song variants by Darwin's finches. *Animal Behaviour*, 73, 833–844.
- Price, T. 2007. *Speciation in Birds*. Greenwood Village, Colorado: Roberts.
- Ptacek, M. B. 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behavioural Processes*, 51, 111–134.
- Qvarnström, A., Haavie, J., Saether, S. A., Eriksson, D. & Pärt, T. 2006. Song similarity predicts hybridization in flycatchers. *Journal of Evolutionary Biology*, 19, 1202–1209.
- Radford, A. N. & Ridley, A. R. 2007. Individuals in foraging groups may use vocal cues when assessing their need for anti-predator vigilance. *Biology Letters*, 3, 249–252.
- Ripmeester, E. A., Mulder, M. & Slabbekoorn, H. 2010. Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. *Behavioral Ecology*, 21, 876–883.
- Ryan, M. J. & Rand, A. S. 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution*, 47, 647–657.
- Sandoval, L. 2011. Male–male vocal interactions in a territorial Neotropical quail: which song characteristics predict a territorial male's response? *Behaviour*, 148, 1103–1120.
- Sandoval, L. & Barrantes, G. 2012. Characteristics of male spot-bellied bobwhite (*Colinus leucopogon*) song during territory establishment. *Journal of Ornithology*, 153, 547–554.
- Sandoval, L. & Mennill, D. J. 2012. Breeding biology of white-eared ground-sparrows (*Melospiza leucotis*), with a description of a new nest type. *Ornitología Neotropical*, 23, 225–234.
- Sandoval, L. & Wilson, D. R. 2012. Local predation pressure predicts the strength of mobbing responses in tropical birds. *Current Zoology*, 58, 781–790.
- Seddon, N. & Tobias, J. A. 2010. Characteristics displacement from the receiver's perspective: species and mate recognition despite convergent signals in suboscine birds. *Proceedings of the Royal Society B*, 277, 2475–2483.
- Slabbekoorn, H. & ten Cate, C. 1997. Stronger territory response to frequency modulated coos in collared doves. *Animal Behaviour*, 135, 879–895.
- Slud, P. 1964. The birds of Costa Rica: distribution and ecology. *Bulletin of American Museum of Natural History*, 128, 1–430.
- Stiles, F. G. & Skutch, A. F. 1989. *Guide to the Birds of Costa Rica*. New York: Cornell University Press.
- Temeles, E. J. 1994. The role neighbours in territorial systems: when are they 'dear enemies'? *Animal Behaviour*, 47, 339–350.
- Templeton, C. N. & Greene, E. 2007. Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. *Proceedings of National Academy of Sciences, U.S.A.*, 104, 5479–5482.
- Tibbetts, E. A. 2002. Visual signals of individual identity in the wasp *Polistes fuscatus*. *Proceedings of the Royal Society of London, Series B*, 269, 1423–1428.

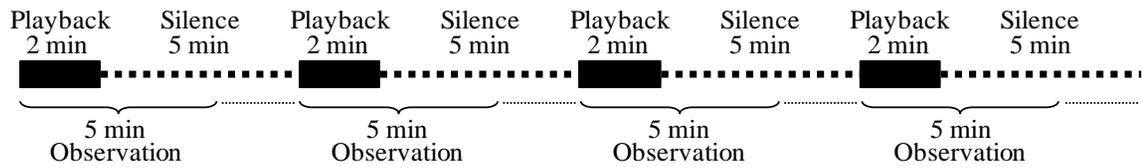
Tibbetts, E. A. & Dale, J. 2004. A socially enforced signal of quality in a paper wasp. *Nature*, 432, 218–222.

Tobias, J. A. & Seddon, N. 2009. Signal design and perception in *Hypocnemis antbirds*: evidence for convergent evolution via social selection. *Evolution*, 63, 3168–3189.

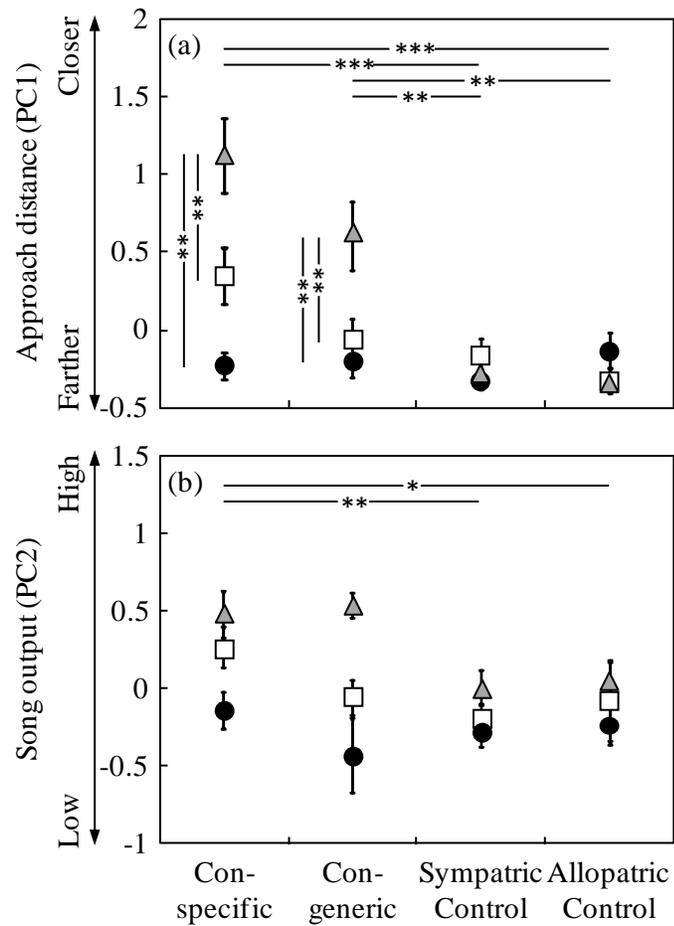
Figures



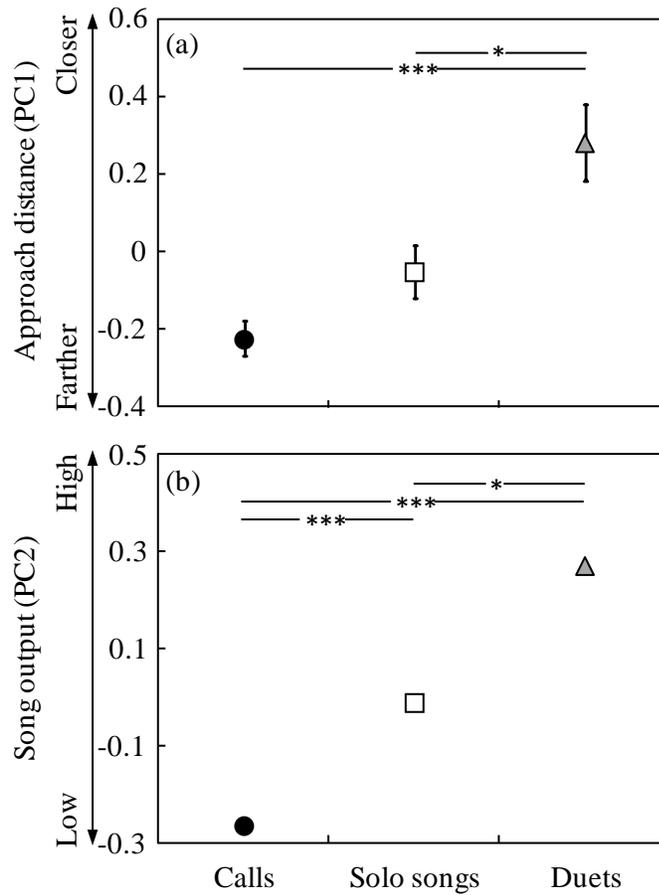
**Figure 6.1.** (Previous page) Sound spectrograms of three types of vocalizations used in the playback experiment to study species recognition in white-eared ground-sparrow. In each spectrogram, a male solo song is shown at the far left, a male–female duet is shown in the centre, and a call is shown at the far right. Conspecific stimuli were white-eared ground-sparrows; congeneric stimuli were Prevost’s ground-sparrows; sympatric control stimuli were plain wrens; and allopatric control stimuli were large-footed finches. White and black bars underscore the contribution of each individual to the duets.



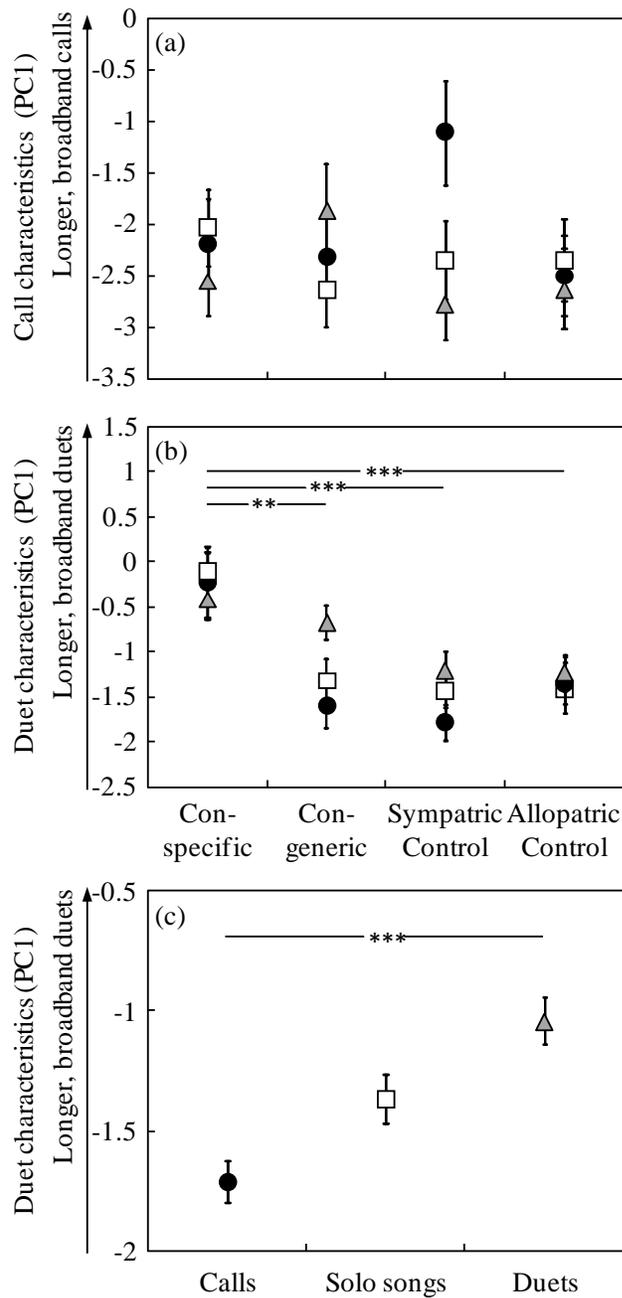
**Figure 6.2.** Schematic representation of the timing of playback trials delivered to white-eared ground-sparrows. During each trial, territorial pairs received four playback treatments (either calls, solos or duets of the four playback species); each pair received three trials on three subsequent days. Playback treatments are represented by black bars and the time between treatments is represented by a thick dotted line. The responses of the subjects were assessed for the first 5 min following the first playback stimulus, and the remaining 2 min were treated as a recovery period.



**Figure 6.3.** Comparison of responses displayed by white-eared ground-sparrow pairs to playback of three types of vocalizations (circles: calls; squares: solo songs; triangles: duets) from four species (conspecific: white-eared ground-sparrow; congeneric: Prevost's ground-sparrow, sympatric control: plain wren; allopatric control: large-footed finch). The responses are measured as principal components scores summarizing (a) variation in approach distance (PC1) and (b) variation in song output (PC2; see text for details). Post hoc statistical differences in response to the four species are represented by horizontal lines; post hoc statistical differences in response to the three types of vocalizations are represented by vertical lines (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ).



**Figure 6.4.** Comparison of responses displayed by white-eared ground-sparrow pairs to playback of three types of vocalizations (circles: calls; squares: solo songs; triangles: duets) averaged across the four species and the two populations. The responses are measured as principal components scores summarizing (a) variation in approach distance (PC1) and (b) variation in song output (PC2; see text for details). Post hoc statistical differences are represented by horizontal lines (\* $P \leq 0.05$ ; \*\*\* $P \leq 0.001$ ).



**Figure 6.5.** Vocal responses of white-eared ground-sparrow pairs to playback of three types of vocalizations (circles: calls; squares: solo songs; triangles: duets) from four species (conspecific: white-eared ground-sparrow; congeneric: Prevost's ground-sparrow; sympatric control: plain wren; allopatric control: large-footed finch). Responses were measured as principal components scores summarizing variation in (a) call and (b, c) duet characteristics (PC1; see text for details). Post hoc statistical differences are represented by horizontal lines (\*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ).

## **Chapter 7**

**Analysis of plumage, morphology, and voice reveal species level differences between Prevost's Ground-sparrows subspecies\***

---

\*This chapter is the outcome of joint research with P-P. Bitton, S. Doucet, and D. Mennill

## Chapter Summary

*Melospiza biarcuata* (Prevost's Ground-sparrow) has traditionally been divided into two allopatric groups based on differences in vocalizations and plumage characteristics: *M. b. biarcuata* and *M. b. hartwegi* in northern Central America and *M. b. cabanisi* in Costa Rica. Since the original description of the species, the relationship between these three subspecies has never been studied using a taxonomic approach. In this study, our objective is to provide the first detailed taxonomic comparison between these three subspecies using an integrative multi-trait approach. We analyzed morphology, plumage patterns, spectral reflectance, and vocalizations of individuals from the three taxa. Our results show that *M. b. cabanisi* can be readily distinguished from the two other subspecies using morphology (*M. b. cabanisi* are smaller), plumage patterns (*M. b. cabanisi* have different facial markings and plumage patches), colour differences (*M. b. cabanisi* have plumage patches that differ in colour and brightness), and vocalizations (*M. b. cabanisi*'s songs and calls are acoustically distinct from those of *M. b. biarcuata*). By contrast, *M. b. biarcuata* and *M. b. hartwegi* were very similar for most traits, supporting previous suggestions that the two northern subspecies should be considered a single subspecies. Our data reveal that the differentiation in phenotypic characteristics between *M. b. cabanisi* and the two northern subspecies is similar in degree to that reported for other complexes of subspecies where species status has been recognized. We argue that *M. b. cabanisi* should be treated as a species separate from *M. biarcuata* and propose that it be called *M. cabanisi*, Cabanis' Ground-Sparrow. These results will contribute to the conservation efforts of Cabanis' Ground-Sparrow, which is endemic to Costa Rica's Central Valley and Turrialba Valley, by bringing focus to conservation policies that preserve ground-sparrow habitat (thickets, shade coffee plantations, and young secondary forest).

## Introduction

The taxonomy of the family Emberizidae, which includes sparrows and buntings, has been the focus of several recent studies at different hierarchical levels. These studies have significantly altered our understanding of the family, where species that were previously considered emberizids have been moved into other families, and species from other families have been moved into Emberizidae (Klicka *et al.* 2000, 2007; Garcia-Moreno *et al.* 2001; Barker *et al.* 2013). For example, recent research has suggested that New World sparrows be classified in a new family called Passerellidae (Barker *et al.* 2013). These studies also evaluated and reorganized species relationships within the family by (1) disentangling species relationship inside problematic genera such as *Aimophila* and *Pipilo* (DaCosta *et al.* 2009), and (2) studying subspecies relationships in depth, such as in *Atlapetes*, *Buarremon*, and *Arremon* (Cadena *et al.* 2007; Cadena & Cuervo 2010). Although these important studies provide us with a better understanding of the relationships between the Emberizidae species, it is still necessary to carry out work in other species and genera to develop a more comprehensive understanding of the species relationships within this family.

The *Melozone* group (Chesser *et al.* 2010), sometimes known as the *Melozone-Pyrgisoma* group (DaCosta *et al.* 2009; Rising 2011), requires careful taxonomic examination. Previous studies have failed to resolve the species relationships within *Melozone* (e.g. DaCosta *et al.* 2009). Furthermore, within this taxonomic group there are unresolved relationships among subspecies. An obvious example is the controversial *M. biarcuata* (Prévost & DesMurs) subspecies complex [*M. b. biarcuata* (Prévost & Des Murs), *M. b. hartwegi* (Brodkorb), and *M. b. cabanisi* (Sclater & Salvin)], which have been argued, at times, to be different species based on anecdotal observations of vocal and plumage differences (Sclater & Salvin 1868; Stiles & Skutch

1989; Howell & Webb 1995; AOU 1998; Sánchez *et al.* 2009). One problematic issue is that the subspecies boundary between *M. b. biarcuata* and *M. b. hartwegi* is not clear (Figure 7.1); in the description of *M. b. hartwegi*, it is referred to as a lowland species of Chiapas, and this was the basis for considering the Mexican birds a separate subspecies compared to the higher elevation birds found further south (Brodkorb 1938). We now know, however, that Mexican birds occur continuously from 100 m to 2500 m along their distribution (Howell & Webb 2004), ruling out the argument that *M. b. hartwegi* is geographically disjunct from *M. b. biarcuata* (Figure 7.1). For this reason, previous investigators have argued that *M. b. hartwegi* is not a valid subspecies, and have grouped them together within *M. b. biarcuata* (Hellmayr 1938; Rising 2011).

Another matter of concern is that the taxonomic status, relationship, and identification of *M. b. cabanisi* have been problematic since this taxon's description. As early as 1868, Sclater and Salvin believed that *M. b. cabanisi* was a species separate from *M. b. biarcuata*, declaring, "it is unfortunate that all the naturalists who have met with specimens of [*M. b. cabanisi*] should have identified it wrongly." Nonetheless, since then, *M. b. cabanisi* has been treated as a subspecies of *M. b. biarcuata* (Rising 2011). Despite the morphological and plumage differences found within *M. biarcuata*, which have been acknowledged since its original description (Sclater & Salvin 1868; Stiles & Skutch 1989; Howell & Webb 1994; Rising 2011), the relationships between the three subspecies have never been studied using a quantitative, taxonomic approach. As a consequence, this group's taxonomic status remains unclear (AOU 1998).

The objective of this investigation is to provide the first detailed and rigorous taxonomic study of the three *M. biarcuata* subspecies and to use an integrative multi-trait approach to evaluate whether the Costa Rican taxon (*M. b. cabanisi*; Figure 7.1) may be better understood as a separate species from the two more northerly taxa, and whether the two northerly taxa should

be considered a single subspecies. *Melospiza b. cabanisi* is geographically isolated from the other two subspecies and is endemic to Costa Rica. It inhabits mainly young dense secondary vegetation and shade coffee plantations of the Central and Turrialba Valleys. The areas covered by these habitats are decreasing at high rates due to urbanization and population growth (Joyce 2006; Sánchez *et al.* 2009; Biamonte *et al.* 2011), adding urgency to the resolution of this taxonomic problem.

## Methods

In this analysis we included characteristics (e.g., morphological, visual, and acoustic) that were consistently present within each subspecies as suggested by Tobias *et al.* (2010), and that they report as important characters to be analyzed. We measured morphology, plumage patterns, and plumage reflectance characteristics of adult specimens of *M. b. biarcuata*, *M. b. hartwegi*, and *M. b. cabanisi*, from the following museums: Museo de Zoología Universidad de Costa Rica, Museo Nacional de Costa Rica, Field Museum of Natural History, University of Michigan Museum of Zoology, and Musée National d'Histoire Naturelle in France (Table S7.1). We also included morphological data collected from two adult male *M. b. cabanisi* captured in Costa Rica. Because the subspecies boundary between *M. b. biarcuata* and *M. b. hartwegi* is not clear (Figure 7.1), our comparison of the two northern subspecies treats the border between Mexico and Guatemala as the boundary between subspecies *hartwegi* and *biarcuata*.

## Morphology

We measured the culmen length (exposed culmen), culmen width and depth (at nares), tarsus and tail length, and wing cord length (unflattened) from 22 *M. b. biarcuata*, 20 *M. b. hartwegi*, and 21 *M. b. cabanisi* museum specimens and the two males captured in the field. All these morphological measurements were uncorrelated within both sexes (females:  $r < 0.63$ ,  $P > 0.21$ ; males:  $r < 0.43$ ,  $P > 0.07$ , for all comparisons). All measurements were taken to the nearest 0.1 mm following the same methods as in Sandoval & Mennill (2013). We conducted multiple analysis of variance (MANOVA) to analyse which morphological measurements are different between the three subspecies. We conducted separate analyses for each sex, because experience in the field has taught us that males are slightly larger than females (Sandoval & Mennill 2013). We used post-hoc tests (pair-wise comparisons) to compare the differences between morphological measurements between subspecies, for all morphological measurements that were different according to the MANOVA.

## Plumage traits and spectrophotometry

We performed a qualitative assessment of plumage patterns by visually evaluating museum specimens (11 *M. b. biarcuata*, 9 *M. b. hartwegi*, and 11 *M. b. cabanisi*). Based on our experience with comparing museum specimens and observing birds in the field, we focused our attention on body regions that showed substantial variation across all specimens, notably the head and the breast, to describe notable differences in plumage patterns across subspecies.

To objectively quantify differences in plumage colouration, we measured plumage colour using reflectance spectrophotometry focusing on ten body regions: throat, breast, belly, undertail coverts, forehead, crown, mantle, pre-ocular spot, cheek (because the cheek of *M. b.*

*biarcuata* fades from black to rust, we targeted both areas of the cheek to obtain the measurements), and the lower-flank (the side of the body, just below the tip of the folded wing). We measured the plumage characteristics for each of these ten body regions for 11 *M. b. biarcuata*, 9 *M. b. hartwegi*, and 11 *M. b. cabanisi* museum specimens. For each body region, we collected five measurements, moving the probe at least 3 mm between measurements, and keeping the probe at a fixed distance perpendicular to the feathers' surface using a rubber stopper (Andersson & Prager 2006). We collected these reflectance data using an Ocean Optics S2000 spectrometer combined with a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA), operated using OOIBase software on a laptop computer. We measured the reflectance as the percentage of light reflected in reference to a Spectralon pure white standard (WS-2, Ocean Optics).

All spectral analyses were conducted using the R package *pavo* (Maia *et al.* 2013). We used a tetrahedral colour-space visual model to compare plumage colouration between the three groups; these visual models allowed us to compare colours while considering how the birds themselves would perceive them, unlike standard colourimetric approaches that consider only the properties of the reflective surface. We compared the characteristics of plumage patches between the three subspecies using the tetrahedral colour-space model (Burkhardt 1989; Goldsmith 1990; Stoddart & Prum 2007) instead of the colour opponency model developed by Vorobyev & Osorio (1998) because the colour opponency model requires more species-specific information, little of which is available for *Melospiza* species. Tetrahedral colour-space allowed us to model the relative stimulation of the retinal photoreceptors using the sensitivity function of each cone separating reflectance characteristics into their chromatic (hue and saturation) and achromatic (brightness) components.

Determining the position of a colour in tetrahedral colour-space required us to make assumptions about: (1) peak sensitivities of all four photoreceptors of the animal's retina; (2) characteristics of the ambient light; and (3) characteristics of the background coloration. (1) We used cone peak sensitivities of the average avian visual system for birds that possess an ultraviolet cone type because most passerines, and the species most closely related to *Melozona*, have an ultraviolet cone type with a peak sensitivity near 370nm (Hart 2001). (2) We used a "forest shade" ambient illumination because these *Melozona* ground-sparrows are found in relatively dense thickets. (3) We used an ideal (wavelength-independent) background because it allows plumage patches to be compared without the influence of a background, which in the case *M. biarcuata*, might change among and within locations. We calculated the achromatic component based on the stimulation of the two longest wavelength cones (Vorobyev & Osorio 1998).

We compared the colours of the same body region between individuals by subspecies using the Euclidean distance separating their three-dimensional coordinates in colour-space. To avoid independence problems, we compared the plumage characteristics of each individual against all others, using a bootstrapping mean of the distance between them according to their index of similarity. Then we used one-way analysis of variance (ANOVA) to determine the mean differences in the chromatic component of body region per subspecies. If we found differences between subspecies, we used pair-wise post-hoc t-tests to compare which subspecies were chromatically different. We compared the brightness value (achromatic component) per body region between subspecies using another ANOVA. For significant differences, we conducted pair-wise post-hoc t-tests to compare which subspecies differed in their brightness.

## Vocal analyses

For our acoustic analyses we used recordings from 11 *M. b. biarcuata* and 32 *M. b. cabanisi*. We were unable to obtain recordings of *M. b. hartwegi* from the field or from sound libraries. We collected recordings in the field using a solid state digital recorder (Marantz PMD661) and a shotgun microphone (Sennheiser ME66/K6). We recorded *M. b. biarcuata* in Guatemala, Suchitepéquez, Reserva Los Tarrales (10°31'N, 91°08'W), and we recorded *M. b. cabanisi* in Costa Rica, Heredia, Getsemaní (10°01'N, 84°06'W) and Calle Tiquisia (10°02'N, 84°04'W), San José, Aserrí (9°51'N, 84°06'W), and Universidad de Costa Rica campus (10°02'N, 84°04'W). We supplemented our recordings with recordings from the private collections of colleagues, from the Macaulay Library of Natural Sounds Cornell Laboratory of Ornithology, and from the Laboratorio de Bioacústica Universidad de Costa Rica (Table S7.2).

We measured the fine-structural properties of both the calls and the male solo songs for both *M. b. biarcuata* and *M. b. cabanisi*. Although these birds produce duets (see Chapter 6), we did not obtain high quality recordings of the duets for the northern subspecies during our field research, and therefore we could not compare this vocalization statistically. For each vocalization we measured the duration (s), the minimum frequency (Hz), the maximum frequency (Hz), and the frequency of maximum amplitude (Hz). For male solo songs we measured the number of elements and the number of unique types of element per song. We collected acoustic measurements using Raven Pro 1.4 sound analysis software (Cornell Lab of Ornithology, Ithaca, NY, USA). We used the following settings in Raven to achieve frequency resolution of 188 Hz and temporal resolution of 5.8 ms: Hann window with 50% overlap and 256 kHz transform size with 16 bit accuracy.

Given that the majority of spectrotemporal measurements in calls were correlated but none were correlated in songs ( $r < 0.56$ ,  $P > 0.09$ ), we conducted a backward stepwise discriminant function analysis (DFA) based on the sample size to select the uncorrelated acoustic measurements that best distinguished *M. b. cabanisi* from *M. b. biarcuata*. We sequentially excluded from the analysis the variable with the lowest  $F$  value, one at the time, and re-ran the analysis after each deletion until we obtained the model with the lowest number of variables and highest correct assignment. We compared the two types of vocalizations between the two subspecies by calculating an average value for each measurement per individual, and using these values as our dependent variables in the DFA. We report the proportion of individuals correctly assigned to their correct taxonomic group based on a jackknife approach for all the analyzed cases. We used pairwise post-hoc t-tests to compare the differences between the acoustic measurements. We used SYSTAT (version 11.00.01; SYSTAT Software, Chicago, IL, USA) for all statistical analyses. Data are reported as means  $\pm$  SE, and all tests are two-tailed.

## Results

### Morphology

We found significant morphological differences between *Melospiza biarcuata cabanisi*, *M. b. biarcuata*, and *M. b. hartwegi* in both sexes. For females, multiple analysis of variance revealed that the best morphological measurement to distinguish between groups was tail length (MANOVA:  $F_{18,31} = 51.27$ ,  $P < 0.001$ ). Post-hoc tests showed that female tail length was significantly longer in *M. b. biarcuata* and *M. b. hartwegi* than in *M. b. cabanisi* (Table 7.1). The other five morphological measurements were similar between females of three subspecies (Table 7.1). For males, the best morphological measurements to distinguish between groups

were tarsus length, tail length, culmen length, and beak height ( $F_{18,102} = 106.82$ ,  $P < 0.001$ ). Post-hoc tests showed that tarsus length, tail length, and culmen length were all longer in male *M. b. biarcuata* and *M. b. hartwegi* than in *M. b. cabanisi* (Table 7.1). The beak height was taller in male *M. b. hartwegi* than in *M. b. biarcuata* and *M. b. cabanisi* (Table 7.1). The other two morphological measurements were similar between males of all subspecies (Table 7.1).

#### Plumage patterns

*Melospiza b. biarcuata* and *M. b. hartwegi* were identical in their plumage patterns, but showed considerable differences in plumage patterns compared to *M. b. cabanisi*. The most marked differences in plumage patterns were on the face and breast (Figure 7.2). Around the eye, *M. b. cabanisi* exhibited a thin white eye ring, a small white postocular spot, and a large white pre-ocular spot, whereas *M. b. biarcuata* and *M. b. hartwegi* exhibited a large white facial mask. *M. b. cabanisi* displayed a black moustache stripe, a white malar stripe, and a black lateral throat stripe; both black stripes were lacking in *M. b. biarcuata* and *M. b. hartwegi*, which instead had a contrasting bicolored auricular patch (black fading to rust) above an incomplete white nape collar. The breast of *M. b. cabanisi* displayed a large circular black patch below the throat whereas *M. b. biarcuata* and *M. b. hartwegi* had no contrasting markings on its white breast. Plumage features were identical for males and females of each subspecies.

#### Plumage colour

Our visual models revealed notable differences in reflectance for some body regions between the three groups (Figure 7.3). Our analyses revealed that the most pronounced differences in

colour were in the cheek and the breast. The two northern subspecies (*M. b. biarcuata* and *M. b. hartwegi*) showed bicolored cheeks (black fading to rust), whereas *M. b. cabanisi* showed rust-coloured cheeks. In the breast, the northern subspecies showed a grey to white breast, but *M. b. cabanisi* showed a black breast spot. For the chromatic component of reflectance, our visual models show that cheek colour ( $F_{2,27} = 8.60$ ,  $P = 0.001$ ) and breast colour ( $F_{2,27} = 5.54$ ,  $P = 0.01$ ) differed between the two northern subspecies (*M. b. biarcuata* and *M. b. hartwegi*) and *M. b. cabanisi* (post-hoc pair-wise comparisons; cheek: *biarcuata-vs-cabanisi*:  $P = 0.001$ ; *hartwegi-vs-cabanisi*:  $P = 0.001$ ; and *biarcuata-vs-hartwegi*:  $P = 0.88$ ; breast: *biarcuata-vs-cabanisi*:  $P = 0.007$ , *hartwegi-vs-cabanisi*:  $P = 0.01$ , and *biarcuata-vs-hartwegi*:  $P = 0.95$ ). For the achromatic component, the brightness of both the breast ( $F_{2,28} = 36.99$ ,  $P < 0.001$ ) and undertail coverts ( $F_{2,28} = 4.43$ ,  $P = 0.02$ ) differed between the two northern subspecies (*M. b. biarcuata* and *M. b. hartwegi*) and *M. b. cabanisi* (breast: *biarcuata-vs-cabanisi*:  $P < 0.001$ , *hartwegi-vs-cabanisi*:  $P < 0.001$ , and *biarcuata-vs-hartwegi*:  $P = 0.42$ ; cheek: *biarcuata-vs-cabanisi*:  $P = 0.048$ , *hartwegi-vs-cabanisi*:  $P = 0.007$ , and *biarcuata-vs-hartwegi*:  $P = 0.30$ ). The brightness of the belly was more similar between *M. b. biarcuata* and *M. b. cabanisi*, in comparison to *M. b. hartwegi* ( $F_{2,28} = 8.18$ ,  $P = 0.001$ ; *biarcuata-vs-cabanisi*:  $P = 0.33$ , *hartwegi-vs-cabanisi*:  $P = 0.004$ , and *biarcuata-vs-hartwegi*:  $P = 0.001$ ). Finally, the brightness of the cheeks was more similar between *M. b. hartwegi* and *M. b. cabanisi*, in comparison to *M. b. biarcuata* ( $F_{2,28} = 4.82$ ,  $P = 0.02$ ; *biarcuata-vs-cabanisi*:  $P = 0.006$ , *hartwegi-vs-cabanisi*:  $P = 0.55$ , and *biarcuata-vs-hartwegi*:  $P = 0.04$ ). For all other body patches our visual models reveal no differences for the chromatic or achromatic component of reflectance ( $P > 0.05$  for all tests).

## Voice

*Melospiza b. cabanisi* exhibited significant acoustic differences in comparison to *M. b. biarcuata* (Figure 7.4). For calls, we found that the fine structural measurement that best distinguished *M. b. biarcuata* calls from *M. b. cabanisi* calls was the maximum frequency (DFA: Wilks'  $\lambda = 0.50$ ,  $F_{1,14} = 14.10$ ,  $P = 0.002$ ). This measurement correctly classified 82% of *M. b. biarcuata* to the correct group (9 of 11) and 100% of the *M. b. cabanisi* in the correct group (5 of 5). In post-hoc analyses of calls, minimum frequency ( $t_{14} = 3.0$ ,  $P = 0.01$ ), maximum frequency ( $t_{14} = 3.8$ ,  $P = 0.002$ ), and frequency of maximum amplitude ( $t_{14} = 3.0$ ,  $P = 0.01$ ), exhibited higher values in *M. b. cabanisi* than in *M. b. biarcuata* (Table 7.2). Call duration was similar between subspecies ( $t_{14} = 1.10$ ,  $P = 0.29$ , Table 7.2).

For male solo songs, we found that the fine structural measurements that best separated *M. b. biarcuata* from *M. b. cabanisi* were song duration, maximum frequency, and frequency of maximum amplitude (DFA: Wilks'  $\lambda = 0.28$ ,  $F_{6,15} = 6.39$ ,  $P < 0.001$ ). Together, these three acoustic measurements correctly classified 100% of *M. b. biarcuata* to the correct group (9 of 9) and 92% of *M. b. cabanisi* to the correct group (12 of 13). Post-hoc tests revealed that *M. b. cabanisi* had higher maximum frequencies ( $t_{20} = 4.6$ ,  $P < 0.001$ ), more song elements ( $t_{20} = 2.2$ ,  $P = 0.04$ ) as well as non-significant tendencies for higher frequencies of maximum amplitude ( $t_{20} = -1.90$ ,  $P = 0.07$ ) and higher minimum frequencies ( $t_{20} = 1.9$ ,  $P = 0.07$ ; Table 7.2). Solo song duration ( $t_{20} = 1.5$ ,  $P = 0.16$ ) and number of element types ( $t_{20} = 1.6$ ,  $P = 0.12$ ) were similar between the subspecies (Table 7.2).

We did not obtain a sufficient number of high quality recordings of the duets of ground-sparrows in the field, in part because their duets are very quiet sounds. We heard northern birds perform duets on a few occasions; to our ear, sounded different from the duets of southern

birds, and based on one recording of intermediate quality, they appear to be structurally different (Figures 7. 4g, h).

## Discussion

Our data show that the allopatric subspecies *Melozone biarcuata cabanisi* in Costa Rica is highly diagnosable from *M. b. biarcuata* and *M. b. hartwegi* in Mexico, Guatemala, El Salvador, and Honduras based on phenotypic characteristics. *Melozone b. cabanisi* can be readily distinguished by morphology, plumage patterns, colour differences, and vocalizations, and is also geographically isolated from the two northern subspecies by more than 500 km. There are no records to date of birds being found in the area between the two parts of their range. Based on our results, which include four different traits, we conclude that the two northern subspecies and the southern subspecies exhibit remarkable differences, pointing towards differentiation on par with many independent species. We also propose based in the high degree of similarities in their three different traits and lack of any defined boundary in the distribution of the two northern subspecies, should be grouped in the same subspecies, *M. b. biarcuata*. Below we explore in more detail each of the differences which point towards a high level of differentiation between the northerly and southerly taxa.

Although the three subspecies inhabit similar habitats (Stiles & Skutch 1989; Howell & Webb 1994; L. Sandoval pers. obs.), a situation which often drives morphology on the same evolutionary path for closely related taxa (Mayr 1976; Ricklefs 2012), we found significant differences in body size between them. Our results for body size agree with initial reports by Sclater & Salvin (1868), which indicated that *M. b. cabanisi* was of smaller size than *M. b. biarcuata*. Interestingly, the differences in body size are consistent with Bergmann's rule, which

states that individuals at higher latitudes have larger body sizes (Meiri 2011). The two northern subspecies, *M. b. biarcuata* and *M. b. hartwegi*, shared more similarity in morphological measurements than either did with *M. b. cabanisi*. These similarities in morphology between the two northern subspecies reinforce the lack of use of morphology to distinguish *M. b. hartwegi* from *M. b. biarcuata* as a subspecies (Brodkorb 1938).

Plumage patterns were markedly different between *M. b. cabanisi* and both of the northern subspecies, allowing unambiguous diagnosis of the northern and southern taxa in the field. For the nine plumage patterns that were different between subspecies, seven were present exclusively in *M. b. cabanisi*, and two in *M. b. biarcuata* and *M. b. hartwegi*. Furthermore, our visual models revealed differences in two chromatic components, and achromatic component, of reflectance (breast and cheeks). The breast in *M. b. cabanisi* showed a black spot lacking in the two north subspecies. The cheek in *M. b. cabanisi* is bicolored (black fading to rust), while in *M. b. biarcuata* and *M. b. hartwegi* it is rufous throughout. In birds, plumage patterns are important as signals of species recognition, especially for territory defence (Matyjasiak 2005). Inside the thick habitats these ground-sparrows inhabit, the breast and facial characteristics are conspicuous body regions. The observed colour and pattern differences in these body regions could therefore be an important component of species recognition. As a consequence, these plumage characteristics may serve as important reproductive isolation barriers, were the northern and southern subspecies ever to come into contact. However, a more detailed experimental study testing these hypotheses is necessary to evaluate the exact function of the plumage traits and colour differences in these taxa.

Our fine structural analyses of vocal characteristics revealed that differences in frequency and the number of elements in male songs allow the discrimination between *M. b.*

*biarcuata* and *M. b. cabanisi* with a very high level of accuracy. In addition, differences in call frequency allowed the proper assignment of subspecies with mean accuracy greater than 90%. Solo songs play an important role in female attraction and territory defence in *Melozone leucotis*, a closely related species (Sandoval & Mennill 2012; Chapter 2), and our field observations suggest that the same may be true in both *M. b. biarcuata* and *M. b. cabanisi*. Therefore, significant differences in the fine structural features of solo songs, such as those we report here, could potentially work as a reproductive barrier for the subspecies, if the subspecies were ever to come into contact. As with male solo songs, calls were highly different between *M. b. biarcuata* and *M. b. cabanisi*, even though these have previously been demonstrated to work mainly as contact and alarm signals in this genus (Chapter 2), suggesting that selective factors beyond sexual selection forces may be influencing the evolution of the acoustic characteristics of vocalizations in the genus *Melozone*.

The northern populations of *Melozone b. biarcuata* are separated from the southern populations of *M. b. cabanisi* by a gap of ca. 550 km. This separation is caused by the disjoint distribution of montane habitats that these two ground-sparrows inhabit (Stiles & Skutch 1989; Howell & Webb 1995; Rising 2011), with one region north of Nicaragua and the other in northern Costa Rica, separated by the Nicaragua depression (Ferrez Weinberg 1992; Marshall & Liebherr 2000). Two significant barriers between the subspecies are humid highlands in southern Honduras and northern Nicaragua, and the dry lowlands of Nicaragua depression, the regions where these birds do not occur (Stiles & Skutch 1989; Howell & Webb 2005). How this separation occurred is unknown; however, climatic oscillation during the Pleistocene may have influenced the current distribution (Haffer 1974; 1987; Webb & Rancy 1996; Barrantes 2009). A phylogeographic analysis will be needed to confirm how long they have been in allopatry.

In conclusion, we found that *M. b. cabanisi* was fully distinguishable from *M. b. biarcuata* and *M. b. hartwegi* based on our comparisons of discrete and continuous phenotypic characteristics used in different and uncorrelated contexts as is expected under the Tobias *et al.* (2010) protocol: locomotion (tarsus), feeding (beak), reproduction and territoriality (solo song and plumage patterns), and alarm communication (calls). Therefore we propose that *M. b. cabanisi* be treated as a different species from their northern counterparts. We suggest that the southern taxon be called *M. cabanisi* (Cabanis' Ground-Sparrow) distinguished from the northern taxa *M. b. biarcuata* and *M. b. hartwegi* (Prevost's Ground-Sparrow). This proposition is supported by similar degree of differences in the phenotypic characteristics reported for the *Arremon torquatus* sparrow complex (Cadena and Cuervo 2010), which are now recognized as different species (Chesser *et al.* 2012; SACC proposal 468 - Remsen *et al.* 2013). We also propose based in the high degree of similarities in their morphology, plumage, and colour patterns, in addition to the lack of any defined boundary in the distribution of the northern subspecies, that *M. b. biarcuata* and *M. b. hartwegi* should be grouped in the same subspecies *M. b. biarcuata*, as has been argued by previous investigators (e.g., Hellmayr 1938; Rising 2011).

Cabanis' Ground-Sparrow is endemic to the Central Valley of Costa Rica (from Atenas and San Ramón in Alajuela province to Paraiso in Cartago province), Turrialba Valley (in the Caribbean side of the country), and the west part of Monteverde mountain range, Guanacaste province, from 500 to 1700 m (Stiles & Skutch 1989; Garrigues & Dean 2007; L. Sandoval pers. obs.). This ground-sparrow inhabits mainly thickets, shade coffee plantations, and young secondary forest (Stiles & Skutch 1989; Garrigues & Dean 2007; Sánchez *et al.* 2009), habitats that are not protected by any conservation laws in Costa Rica. The intense levels of urbanization in Costa Rica's Central Valley endangers these thicket habitats and coffee plantations, reducing the total coverage of this habitat and fragmenting what habitat remains (Joyce 2006; Biamonte

*et al.* 2011). If urbanization of thicket and shade coffee habitat continues at its current pace, Cabanis' Ground-sparrow faces an uncertain future, potentially making this species one of the more endangered bird species in Costa Rica. This endemic taxon brings to light the importance of conserving early successional habitats.

## References

- American Ornithologists' Union (AOU) (1998) *Check list of North American birds, 7th edn.* American Ornithologists' Union, Washington, 829 pp.
- Andersson, S. & Prager, M. (2006) Quantifying colors. *In:* Hill, G.E. & McGraw, K.J. (Eds.), *Bird coloration. Volume 1: Mechanisms and measurements.* Harvard University Press, Cambridge, pp. 41–89.
- Barker, F.K., Burns, K.J., Klicka, J., Lanyon, S.M. & Lovette, I.J. (2013) Going to extremes: contrasting rates of diversification in a recent radiation of New World Passerine birds. *Systematic Biology*, 62, 298–320.  
<http://dx.doi.org/10.1093/sysbio/sys094>
- Barrantes, G. (2009) The role of historical and local factors in determining species composition of the highland avifauna of Costa Rica and Panamá. *Revista de Biología Tropical*, 57, 333–349.
- Biamonte, E., Sandoval, L., Chacón, E. & Barrantes, G. (2011) Effect of urbanization on the avifauna in a tropical metropolitan area. *Landscape Ecology*, 26, 183–194.  
<http://dx.doi.org/10.1007/s10980-010-9564-0>
- Brodkorb, P. (1938) New birds from the district of Soconusco, Chiapas. *Occasional Paper Museum Zoology University of Michigan*, 369, 1–8.
- Burkhardt, D. (1989) UV vision: a bird's eye view of feathers. *Journal of Comparative Physiology A*, 164, 787–796.
- Cadena, C.D., Klicka, J. & Ricklefs, R.E. (2007) Evolutionary differentiation in the Neotropical montane region: molecular phylogenetics and phylogeography of *Buarremon* brush-Finches (Aves, Emberizidae). *Molecular Phylogenetics Evolution*, 44, 993–1016.  
<http://dx.doi.org/10.1016/j.ympev.2006.12.012>
- Cadena, C.D. & Cuervo, A.M. (2010) Molecules, ecology, morphology, and songs in concert: how many species is *Arremon torquatus* (Aves: Emberizidae)? *Biological Journal of Linnean Society*, 99, 152–176.  
<http://dx.doi.org/10.1111/j.1095-8312.2009.01333.x>
- Chesser, R.T., Banks, R.C., Barker, F.K., Cicero, C., Dunn, J.L., Kratter, A.W., Lovette, I.J., Rasmussen, P.C., Remsen, Jr. J.V., Rising, J.D., Stotz, D.F. & Winker, K. (2010) Fifty-first supplement to the American Ornithologists' Union check-list of North American birds. *Auk*, 127, 726–744.  
<http://dx.doi.org/10.1525/auk.2010.127.3.726>
- Chesser, R.T., Banks, R.C., Barker, F.K., Cicero, C., Dunn, J.L., Kratter, A.W., Lovette, I.J., Rasmussen, P.C., Remsen, Jr. J.V., Rising, J.D., Stotz, D.F. & Winker, K. (2012) Fifty-third supplement to the American Ornithologists' Union check-list of North American birds. *Auk*, 129, 573–588.  
<http://dx.doi.org/10.1525/auk.2012.129.3.573>
- Chesser, R.T., Banks, R.C., Barker, F.K., Cicero, C., Dunn, J.L., Kratter, A.W., Lovette, I.J., Rasmussen, P.C., Remsen, Jr. J.V., Rising, J.D., Stotz, D.F. & Winker, K. (2013) Fifty-fourth

- supplement to the American Ornithologists' Union check-list of North American birds. *Auk*, 130, 558–572.  
<http://dx.doi.org/10.1525/auk.2013.130.3.1>
- DaCosta, J.M., Spellman, G.M., Escalante, P. & Klicka, J. (2009) A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *Journal of Avian Biology*, 40, 206–216.  
<http://dx.doi.org/10.1111/j.1600-048X.2009.04514.x>
- Ferrez Weinberg, R. (1992) Neotectonic development of western Nicaragua. *Tectonics*, 11, 1010–1017.  
<http://dx.doi.org/10.1029/92TC00859>
- Garrigues, R. & Dean, R. (2007) *The birds of Costa Rica, a field guide*. Zona Tropical Publication, San José, Costa Rica, 416 pp.
- García-Moreno, J., Ohlson, J. & Fjeldså, J. (2001) MtDNA sequences support monophyly of *Hemispingus* tanagers. *Molecular Phylogenetics Evolution*, 21, 424–435.  
<http://dx.doi.org/10.1006/mpev.2001.1027>
- Goldsmith, T.H. (1990) Optimization, constraint, and history in the evolution of eyes. *Quarterly Review of Biology*, 65, 281–322.
- Haffer, J. (1974) *Avian speciation in tropical South America, with a systematic survey of the toucans (Ramphastidae) and jacamars (Galbulidae)*. Publications of the Nuttall Ornithological Club, Cambridge, 390 pp.
- Haffer, J. (1987) Quaternary history of tropical America. In: Whitmore, T.C. & Prance, G.T. (Eds.), *Biogeography and Quaternary history in tropical America*. Clarendon Press, Oxford, pp. 1–18.
- Hart, N.S. (2001) Variation in cone photoreceptor abundance and the visual ecology of birds. *Journal of Comparative Physiology A*, 187, 685–697.  
<http://dx.doi.org/10.1007/s00359-001-0240-3>
- Hart, N.S., Partridge, J. & Cuthill, I. (1998) Visual pigment, oil droplets and cone photoreceptor distribution in the European Starling (*Sturnus vulgaris*). *Journal of Experimental Biology*, 201, 1433–1446.
- Hellmayr, C.E. (1938) Catalogue of birds of the Americas and the adjacent islands. *Field Museum Natural History*, 430, 1–662.
- Howell, S.N.G. & Webb, S. (1995) *A guide to the birds of Mexico and northern Central America*. Oxford University Press, New York, 1010 pp.
- Joyce, A.T. (2006) *Land use change in Costa Rica: 1996-2006, as influenced by social, economic, political, and environmental factors*. Litografía e imprenta LIL, San José, Costa Rica, 272 pp.
- Klicka, J., Burns, K. & Spellman, G.M. (2007) Defining a monophyletic Cardinalini: a molecular perspective. *Molecular Phylogenetics Evolution*, 45, 1014–1032.  
<http://dx.doi.org/10.1016/j.ympev.2007.07.006>

- Klicka, J., Johnson, K.P. & Lanyon, S.M. (2000) New World nine-primaried oscine relationships: Constructing a mitochondrial DNA framework. *Auk*, 117, 321–336.  
[http://dx.doi.org/10.1642/0004-8038\(2000\)117\[0321:NWNPOR\]2.0.CO;2](http://dx.doi.org/10.1642/0004-8038(2000)117[0321:NWNPOR]2.0.CO;2)
- Maia, R., Eliason, C., Bitton, P.-P., Doucet, S. & Shawkey, M. (2013) pavo: an R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution*, 4, 906–913.  
<http://dx.doi.org/10.1111/2041-210X.12069>
- Maier, E.J. (1994) Ultraviolet vision in a passeriform bird: from receptor spectral sensitivity to overall spectral sensitivity in *Leiothrix lutea*. *Vision Research*, 34, 1415–1418.  
[http://dx.doi.org/10.1016/0042-6989\(94\)90141-4](http://dx.doi.org/10.1016/0042-6989(94)90141-4)
- Marshall, C.J. & Liebherr, J.K. (2000) Cladistic biogeography of the Mexican transition zone. *Journal of Biogeography*, 27, 203–216.  
<http://dx.doi.org/10.1046/j.1365-2699.2000.00388.x>
- Matyjasiak, P. (2005) Birds associate species-specific acoustic and visual cues: recognition of heterospecific rivals by blackcaps. *Behavioral Ecology*, 16, 467–471.  
<http://dx.doi.org/10.1093/beheco/ari012>
- Mayr, E. (1976) *Evolution and diversity of life*. Belknap Press, Cambridge, 736 pp.
- Meiri, S. (2011) Bergmann's rule—what's in a name? *Global Ecology and Biogeography*, 20, 203–207.  
<http://dx.doi.org/10.1111/j.1466-8238.2010.00577.x>
- Remsen, Jr. J.V., Cadena, C.D., Jaramillo, A., Nores, M., Pacheco, J.F., Pérez-Emán, J., Robbins, M.B., Stiles, F.G., Stotz, D.F. & Zimmer, K.J. (2013) A classification of the bird species of South America. American Ornithologists' Union: Version July 2013. Available from:  
<http://www.museum.lsu.edu/~Remsen/SACCBaseline.html> (Accessed 12 November 2013)
- Ricklefs, R.E. (2012) Species richness and morphological diversity of passerine birds. *Proceedings of the National Academy of Sciences*, 109, 14482–14487.  
<http://dx.doi.org/10.1073/pnas.1212079109>
- Rising, J.D. (2011) Family Emberizidae (buntings and New World sparrows). In: del Hoyo, J., Elliot, A. & Christie, D. (Eds.), *Handbook of the birds of the world, Volume 16 tanagers to New World blackbirds*. Lynx Edicions, Barcelona, pp 428–683.
- Sánchez, J.E., Criado, J., Sánchez, C. & Sandoval, L. (2009) Costa Rica. In: Devenish, C., Díaz Fernández, D.F., Clay, R.P., Davison, I.J. & Yépez Zabala, I. (Eds.), *Important bird areas of Americas: priority sites for biodiversity conservation*. BirdLife International, Quito, Ecuador, pp. 149–156.
- Sandoval, L. & Mennill, D.J. (2012) Breeding biology of White-eared Ground-sparrow (*Melospiza leucotis*), with a description of a new nest type. *Ornitología Neotropical*, 23, 225–234.
- Sandoval, L. & Mennill, D.J. (2013) Morphometric measurements permit accurate sexing of three species of Mesoamerican ground-sparrow (Genus: *Melospiza*). *Wilson Journal of Ornithology*, 125, 471–478.

- Sclater, P.L. & Salvin, O. (1868) Descriptions of new or little-known American birds of families Fringillidae, Oxyrhamphidae, Bucconidae, and Strigidae. *Proceedings of Zoological Society London*, 36, 322–329.
- Stiles, F.G. & Skutch, A.F. (1989) *A guide to the birds of Costa Rica*. Cornell University Press, Ithaca, 632 pp.
- Stoddard, M.C. & Prum, R.O. (2008) Evolution of avian plumage color in a tetrahedral color-space: a phylogenetic analysis of New World buntings. *American Naturalist*, 171, 755–776.  
<http://dx.doi.org/10.1086/587526>
- Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D. & Collar, N.J. (2010) Quantitative criteria for species delimitation. *Ibis*, 152, 724–746.  
<http://dx.doi.org/10.1111/j.1474-919X.2010.01051.x>
- Vorobyev, M. & Osorio, D. (1998) Receptor noise as a determinant of colour thresholds. *Proceedings of Royal Society London B*, 265, 351–358.  
<http://dx.doi.org/10.1098/rspb.1998.0302>
- Webb, S.D. & Rancy, A. (1996) Late Cenozoic evolution of the Neotropical mammal fauna. *In*: Jackson, J.B.C., Budd, A.F. & Coates, A.G. (Eds.), *Evolution and environment in tropical America*. University of Chicago Press, Chicago, pp. 335–358.

## Tables

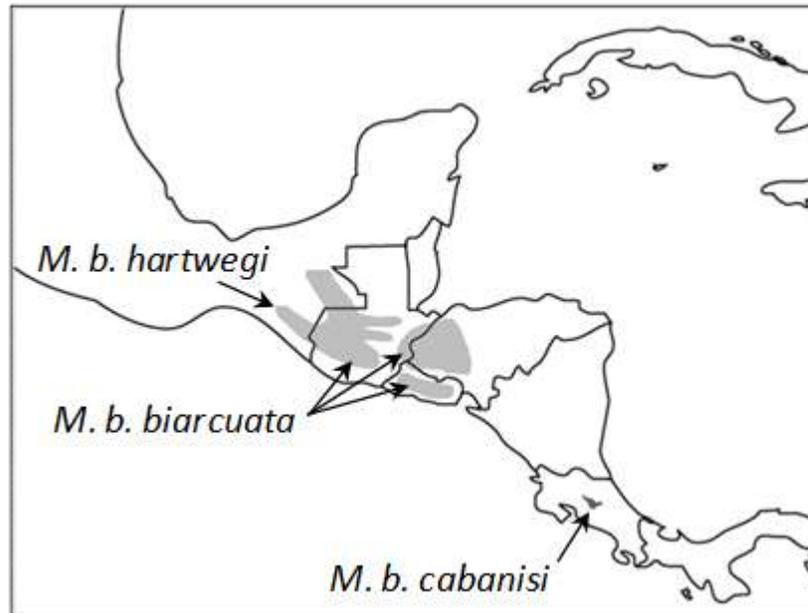
**Table 7.1** Mean ( $\pm$  SE) morphometric measurements by sex in three *Melospiza biarcuata* subspecies. Bold text indicates significant differences between subspecies; brackets in letters show the results of pair-wise post-hoc tests (subspecies with different letters are statistically different).

<b>Females</b>	<i>M. b. biarcuata</i>	<i>M. b. hartwegi</i>	<i>M. b. cabanisi</i>
Tarsus (mm)	24.58 $\pm$ 0.51	24.11 $\pm$ 0.16	23.89 $\pm$ 0.31
<b>Tail length (mm)</b>	<b>60.2 <math>\pm</math> 1.02 (a)</b>	<b>62.3 <math>\pm</math> 0.81 (a)</b>	<b>56.66 <math>\pm</math> 1.21 (b)</b>
Wing cord length (mm)	65.82 $\pm$ 1.82	64.28 $\pm$ 0.87	67.20 $\pm$ 0.86
Culmen length (mm)	12.60 $\pm$ 0.28	13.15 $\pm$ 0.19	12.31 $\pm$ 0.32
Beak width (mm)	8.14 $\pm$ 0.35	8.68 $\pm$ 0.18	7.95 $\pm$ 0.23
Beak depth (mm)	8.20 $\pm$ 0.35	7.70 $\pm$ 0.16	8.30 $\pm$ 0.20
<b>Males</b>			
<b>Tarsus (mm)</b>	<b>24.9 <math>\pm</math> 0.18 (a)</b>	<b>25.14 <math>\pm</math> 0.36 (a)</b>	<b>23.9 <math>\pm</math> 0.27 (b)</b>
<b>Tail length (mm)</b>	<b>65.94 <math>\pm</math> 0.91 (a)</b>	<b>67.25 <math>\pm</math> 0.69 (a)</b>	<b>59.97 <math>\pm</math> 0.80 (b)</b>
Wing cord length (mm)	69.52 $\pm$ 0.62	69.36 $\pm$ 0.53	68.41 $\pm$ 0.83
<b>Culmen length (mm)</b>	<b>13.04 <math>\pm</math> 0.15 (a)</b>	<b>13.55 <math>\pm</math> 0.14 (b)</b>	<b>12.64 <math>\pm</math> 0.12 (c)</b>
Beak width (mm)	7.94 $\pm$ 0.15	8.38 $\pm$ 0.16	8.30 $\pm$ 0.15
<b>Beak depth (mm)</b>	<b>8.33 <math>\pm</math> 0.10 (a)</b>	<b>8.91 <math>\pm</math> 0.09 (b)</b>	<b>8.33 <math>\pm</math> 0.15 (a)</b>

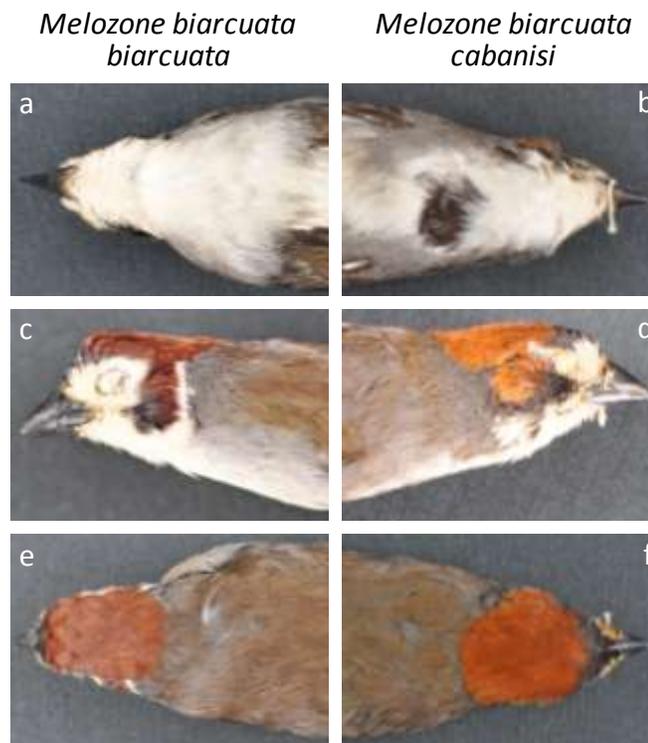
**Table 7.2** Mean ( $\pm$  SE) values of male solo song and call fine acoustic measurements by sex and *Melospiza biarcuata* subspecies. Bold text variables indicate significant differences between subspecies.

<b>Solo songs</b>	<i>M. b. biarcuata</i>	<i>M. b. cabanisi</i>
Number of elements	6.06 $\pm$ 0.38	7.91 $\pm$ 0.66
Number of unique element types	3.21 $\pm$ 0.22	3.60 $\pm$ 0.13
<b>Duration (s)</b>	<b>1.76 <math>\pm</math> 0.22</b>	<b>1.46 <math>\pm</math> 0.08</b>
Minimum frequency (Hz)	2277 $\pm$ 81	2814 $\pm$ 225
<b>Maximum frequency (Hz)</b>	<b>8582 <math>\pm</math> 360</b>	<b>10460 <math>\pm</math> 234</b>
<b>Frequency of maximum amplitude (Hz)</b>	<b>4726 <math>\pm</math> 376</b>	<b>5456 <math>\pm</math> 188</b>
<b>Calls</b>		
Duration (s)	1.33 $\pm$ 0.28	0.81 $\pm$ 0.32
<b>Minimum frequency (Hz)</b>	<b>3248 <math>\pm</math> 444</b>	<b>5535 <math>\pm</math> 570</b>
<b>Maximum frequency (Hz)</b>	<b>9080 <math>\pm</math> 433</b>	<b>11719 <math>\pm</math> 394</b>
<b>Frequency of maximum amplitude (Hz)</b>	<b>5212 <math>\pm</math> 324</b>	<b>6943 <math>\pm</math> 456</b>

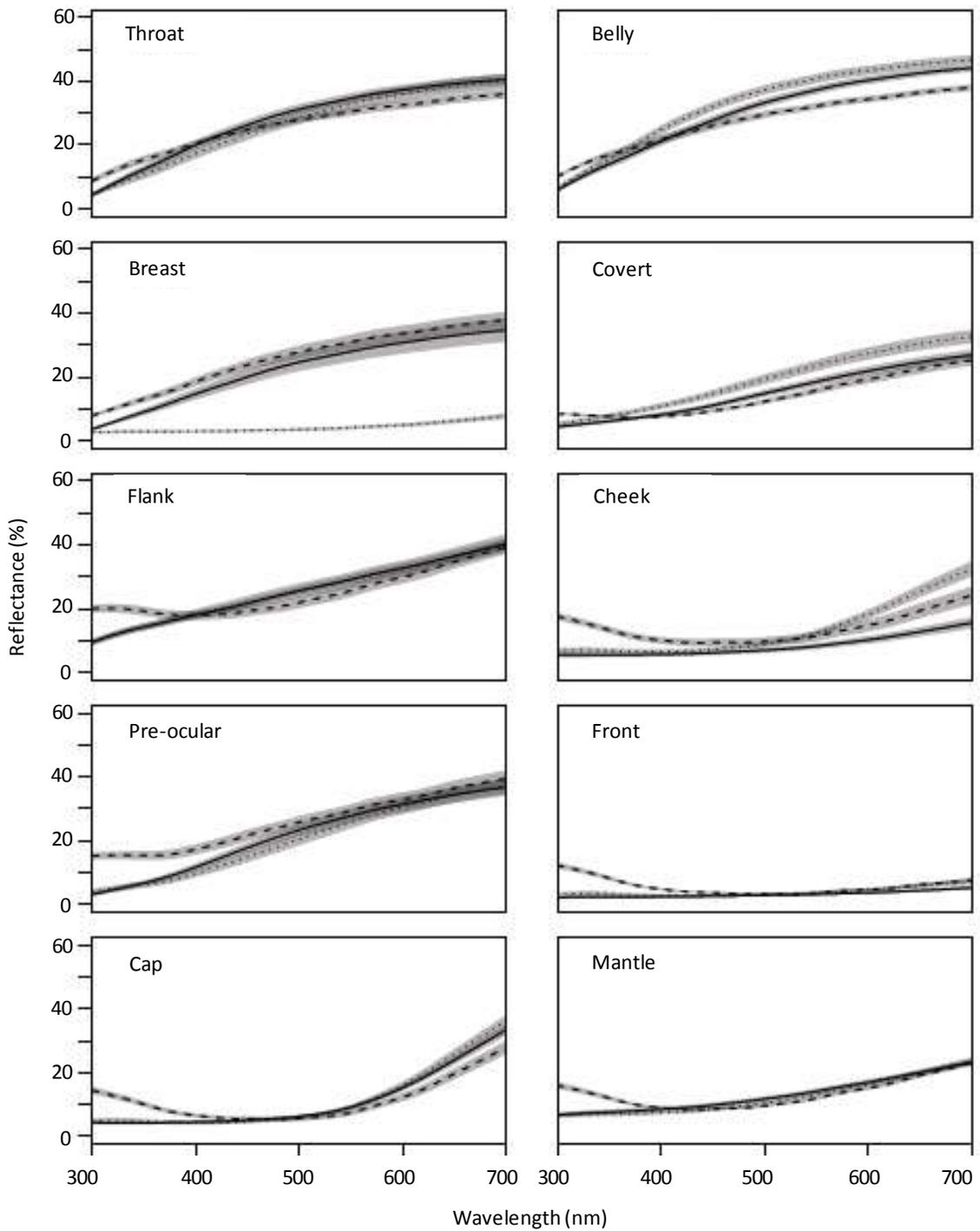
**Figures**



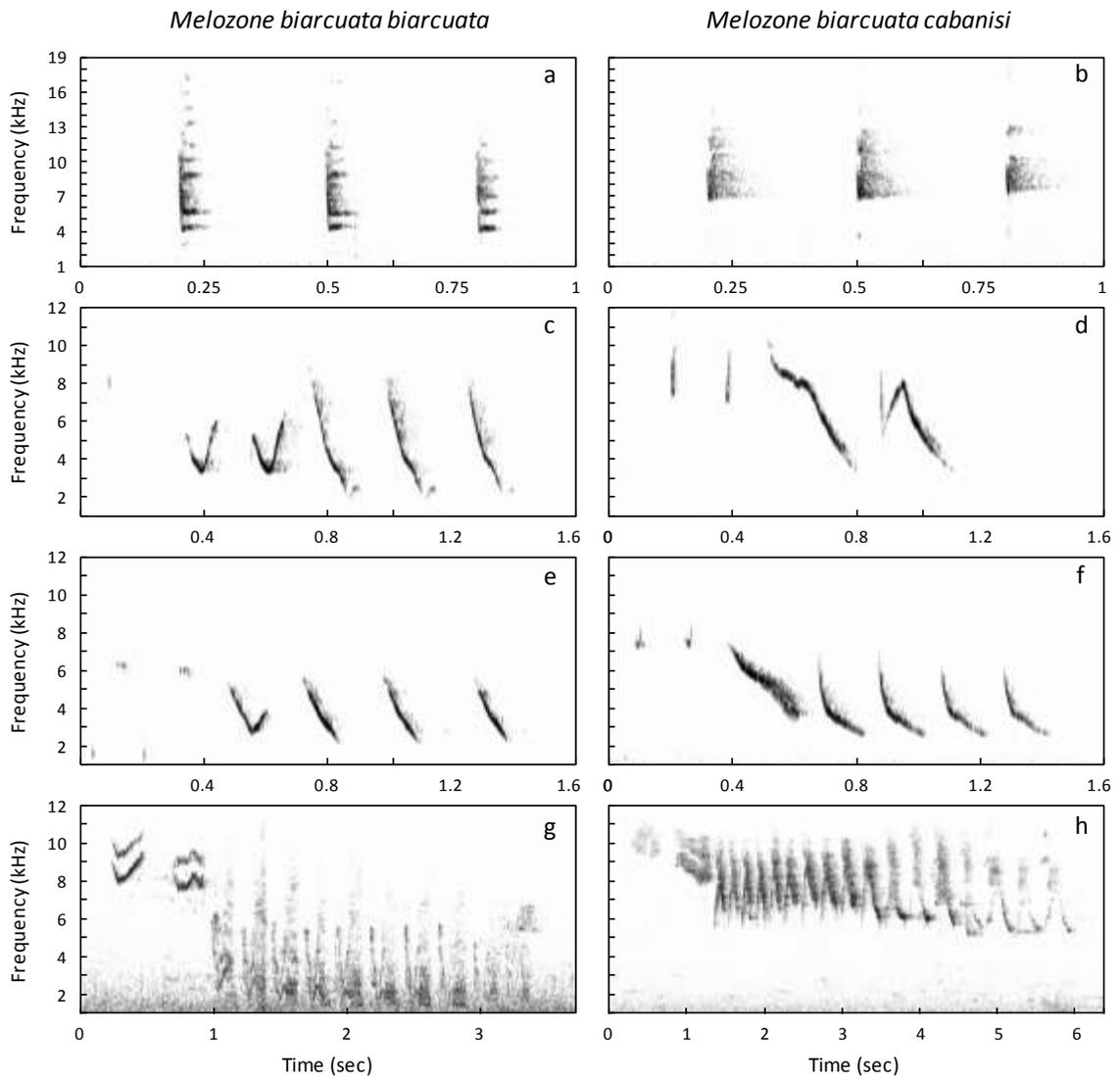
**Figure 7.1.** Distribution of the *Melospiza biarcuata* subspecies from Mexico to Costa Rica. The distribution of *M. b. hartwegi* and *M. b. biarcuata* is continuous. The southern subspecies, *M. b. biarcuata*, is separated by approximately 550 km from the northern subspecies by the Nicaraguan depression.



**Figure 7.2.** Plumage colour and pattern differences between *M. b. biarcuata* (left) in *M. b. cabanisi* (right). Photographs were taken under the same light conditions at the Musée National d'Histoire Naturelle in Paris, France. The top row shows the differences in breast and throat patterns, the middle row shows differences in head patterns, and the bottom row shows differences in crowns.



**Figure 7.3.** Average reflectance spectra for ten body regions measured in 11 *M. b. biarcuata*, 9 *M. b. hartwegi*, and 11 *M. b. cabanisi*. The grey area around each line represents standard error. Solid lines show *M. b. biarcuata*; dashed lines show *M. b. hartwegi*; and dotted lines show *M. b. cabanisi*.



**Figure 7.4.** Sound spectrograms of calls (a, b), male solo songs (c - f), and duets (g, h) of *M. b. biarcuata* (left) and *M. b. cabanisi* (right). See text for a detailed explanation of the differences between subspecies.

## **Chapter 8**

### **General Discussion**

## **Significance**

With this body of research, I provided the first description of the vocal repertoire for the Mesoamerican ground-sparrows in the genus *Melozona*, expanding our knowledge of the diversity of vocalizations in this genus specifically, and the sparrow family (Emberizidae) more generally. Our knowledge of tropical sparrows is very limited (Rising 2011), and my research has helped to address this limitation. For example, I provide the evidence about how unusual the diel pattern of the White-eared and Rusty-crowned Ground-sparrows is in comparison to the majority of the studied bird species (Staicer et al. 1996). Both studied species showed a very high output at dawn followed by low-or-absent output throughout the day. Meanwhile, the majority of the species maintain medium level of vocal output production throughout the rest of the day, including a small peak at sunset (Staicer et al. 1996). I also provide evidence for the production of duets with vocalizations that differ in acoustical structure from vocalizations used for solo songs, as occurs in the majority of duetting species (Mann et al. 2003, Mennill and Vehrencamp 2005, Logue 2006). The occurrence of this particular type of duet vocalizations appears to be common inside several closely related sparrows species that inhabit in the Neotropical and temperate habitats (Benedict and McEntee 2009, Illes and Yunes-Jimenez 2009).

I have provided evidence for how habitat, spatial distribution, and intra- and interspecific competition may or may not influence the evolution of the characteristics of different categories of vocal signals (calls, solo song, and duets) and their perception. This study is important because it provides evidence that the environment is probably not the main cause to drive all the adaptations in vocal characteristics, and therefore it is necessary to be cautious in

the use of this factor to explain the variation between vocal signals and between species. I also show how different categories of vocal signals are under different selective pressures and therefore that it is important to compare within species how each vocal signal has evolved.

Finally, I provided new information about the taxonomic status of three controversial subspecies in the *Melospiza* genus (Sclater and Salvin 1868; Stiles and Skutch 1989; Howell and Webb 1995; AOU 1998; Sánchez et al. 2009), using a phenotypic multi trait comparison of uncorrelated characters. This approach is a very valuable tool to analyze taxonomic relationships between taxa where the genetic data is lacking. However, for more accurate results, it is highly recommended to include samples from several locations where the taxa occur to have a broad representation of the variation, to avoid biasing the results to the extremes of a clinal variation. My investigations enhance our understanding of the taxonomy inside the sparrows, family Emberizidae, which has recently been the focus of more scrutiny (Klicka et al. 2000, 2007, Garcia-Moreno et al. 2001, Barker et al. 2013).

### **Suggestions for future research**

Future investigations can build upon my dissertation research, expand on these findings, and clarify some of the new ideas that I have presented in these data chapters. Relative to songs and duets, the calls of tropical birds are poorly understood. Future research on the behaviour, ecology, and evolution of calls would help to provide a better understanding of the function and transmission properties of these simple vocalizations. Future research on the calls of ground-sparrows needs should focus on evaluating the function of calls (possibly involving playback), providing more detailed observations of the behavioural context of calls, and exploring how

different call rates may encode different types of messages (as in Templeton et al. 2005, Wilson and Mennill 2011 for example). Future research should explore individual distinctiveness of calls and the transmission properties of the two call types that appear to be common among the three species I studied here.

My research revealed that ground-sparrow duets are produced by males and females singing different types of vocalizations than solo songs (chapter 2 and 3). This is an uncommon behaviour among duetting bird species; the majority of duetting species produce duets using the same types of vocalizations used in solo songs (e.g. Mann et al. 2003, Mennill and Vehrencamp 2005, Logue 2006). To the best of my best knowledge, none of the hypotheses proposed for duet function (Hall 2004, 2009) have been tested in species that perform duets with different vocalizations than their solo songs. This is therefore a new avenue for investigating duetting; in particular, given that the Acoustic Contact Hypothesis predicts that duets are used for individual identity in mating contact, it would be worthwhile to investigate whether each individual's duet contribution includes individually-distinctive characteristics, as one would predict under the Acoustic Contact Hypothesis.

Future field studies and genetic studies are needed to advance our understanding of extra-pair copulations and extra-pair fertilizations in ground-sparrows. My research demonstrated that paired male ground-sparrows produce almost all of their solo songs during the first hour of the day, throughout the breeding season, from song posts that were often near territory edges. In other bird species, this type of singing behaviour has been associated with an increase in the probability of extra pair copulations for both sexes with neighbouring individuals (Gibbs et al. 1990, Richardson and Burke 2001, Mennill et al. 2004). If *Melospiza* have extra pair

copulations, as do the majority of birds (Griffiths et al. 2002), including tropical birds (Macedo et al. 2008), then we could come to understand the function of male solo songs, and the diel pattern of the timing of their production. If they do not have extra pair copulations, however, we would interpret the pattern I presented of heightened male solo song vocal output in the early morning as territorial defence.

The occurrence of solo song repertoires among bird species has been studied in detail, but the idea of individual distinctiveness based on this characteristic has been investigated rarely, although it is believed that it may play an important role in individual recognition (Hartshorne 1956, Krebs 1977, Hultsch and Todt 1981, Searcy and Andersson 1986). Therefore, studies that attempt to evaluate individual distinctiveness need to take into account this higher-order cue of individuality and its consistency over time, with the objective of evaluating whether the pattern I have documented here stands up among longer recording periods and in other species. Furthermore, playback studies would be helpful for determining whether birds actually use the pattern of repertoire delivery as a cue of individual distinctiveness. This experiment could involve playback of stimuli that mimic a familiar neighbour's pattern of repertoire delivery, and an unfamiliar non-neighbour's pattern of repertoire delivery. If males are using pattern of repertoire delivery to recognize between neighbours, I would expect to see a stronger response to the non-neighbour treatment than the neighbour treatment.

The results of my playback experiment suggest that ground-sparrows can discriminate between conspecific and congeneric competitors based on the characteristics of their vocalizations alone. However, the observed responses to playback were subtle, and therefore a more detailed experimental study including visual signals associated with auditory signals is

highly recommended. This would help to evaluate whether multiple signal modalities facilitate differentiation between conspecific and congeneric competitors. All species I have studied here have very distinctive facial markings, and therefore tests that involve taxonomic models that follow the design of Searcy and collaborators (2006), for example, would allow us to evaluate if these ground-sparrows also use visual signals to distinguish between conspecific and congeneric competitors. I also recommend avoiding the use of playback in quick succession to answer these types of questions, because the recovery time in those playbacks for focal species perhaps is not enough, and the successive responses may carry the effect of the previous stimuli.

Finally, an area of particular importance is a future phylogenetic analysis, evaluating the genetic relationships between all species and subspecies in this genus, which would aid in understanding the evolutionary origins of the genus *Melospiza*. A phylogenetic analysis would lay foundations for future comparative studies on the divergence of vocalizations, plumage patterns, and habitat use patterns. Recent genetic work has restructured the genus *Melospiza* by showing that four northern species formerly considered *Pipilo* are actually part of *Melospiza* (DaCosta et al. 2009, Chesser et al. 2010). I predict that genetic analyses will confirm the results of my analysis of vocalization, plumage patterns, and colour spectrophotometric analyses, revealing that Cabanis' Ground-sparrow is a distinct species from Prevost's Ground-sparrow.

## **Conclusion**

My dissertation provides a body of evidence describing the vocalizations of tropical ground-sparrows and exploring how different factors influence or constrain the divergence of vocal

signals. Furthermore, my dissertation sheds light on the importance of including different vocalization categories while conducting comparative studies in order to better understand the factors affecting vocal evolution. Finally, I provided evidence on the use of phenotypic characteristics to disentangle problematic taxonomic relationships between closely related subspecies and species.

## References

- American Ornithologists' Union (AOU). 1998. Check list of North American birds, 7<sup>th</sup> edition. American Ornithologists' Union, Washington.
- Barker FK, Burns KJ, Klicka J, Lanyon SM, Lovette IJ. 2013. Going to extremes: contrasting rates of diversification in a recent radiation of New World Passerine birds. *Syst. Biol.* 62:298-320.
- Benedict L, McEntee JP. 2009. Context, structural variability and distinctiveness of California Towhee (*Pipilo crissalis*) vocal duets. *Ethology* 115:77-86.
- Chesser RT, Banks RC, Barker FK, Cicero C, Dunn JL, Kratter AW, Lovette IJ, Rasmussen PC, Remsen JV Jr, Rising JD, Stotz DF, Winker K. 2010. Fifty-First supplement to the American Ornithologists' Union check-list of North American birds. *Auk* 127:726-744.
- DaCosta JM, Spellman GM, Escalante P, Klicka J. 2009. A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *J. Avian Biol.* 40:206-216.
- García-Moreno J, Ohlson J, Fjeldså J. 2001. MtDNA sequences support monophyly of *Hemispingus* tanagers. *Mol. Phyl. Evol.* 21:424-435.
- Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ. 1990. Realized reproductive success of polygynous Red-winged Blackbirds revealed by DNA markers. *Science* 250:1394-1397.
- Griffith SC, Owens IP, Thuman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* 11:2195-2212.
- Hall ML. 2004. A review of hypotheses for the functions of avian duetting. *Behav. Ecol. Sociobiol.* 55:415-430.
- Hall ML. 2009. A review of vocal duetting in birds. *Adv. Stud. Behav.* 40:67-121.
- Hartshorne C. 1956. The monotony-threshold in singing birds. *Auk* 83:176-192.
- Howell SNG, Webb S. 1995. A guide to the birds of Mexico and northern Central America. Oxford University Press, New York.
- Hultsch H, Todt D. 1981. Repertoire sharing and songpost distance in Nightingales (*Luscinia megarhynchos* B). *Behav. Ecol. Sociobiol.* 8:183-188.
- Illes AE, Yunes-Jimenez L. 2009. A female songbird out-sings male conspecifics during simulated territorial intrusions. *Proc. R. Soc. B* 276:981-986.
- Klicka J, Burns K, Spellman GM. 2007. Defining a monophyletic Cardinalini: a molecular perspective. *Mol. Phyl. Evol.* 45:1014-1032.
- Klicka J, Johnson KP, Lanyon SM. 2000. New World nine-primaried oscine relationships: Constructing a mitochondrial DNA framework. *Auk* 117:321-336.
- Krebs JR. 1977. The significance of song repertoires: the Beau Geste hypothesis. *Anim. Behav.* 25:475-478.
- Logue DM. 2006. The duet code of the female black-bellied wren. *Condor* 108:326-335.

- Macedo RH, Karubian J, Webster MS. 2008. Extrapair paternity and sexual selection in socially monogamous birds: are tropical birds different? *Auk* 125:769-777.
- Mann NI, Marshall-Ball L, Slater PJB. 2003. The complex song duet of the Plain Wren. *Condor* 105:672-682.
- Mennill DJ, Vehrencamp SL. 2005. Sex differences in the singing and duetting behavior of neotropical Rufous-and-white Wrens (*Thryothorus rufalbus*). *Auk* 122:175-186.
- Mennill DJ, Ramsay SM, Boag PT, Ratcliffe LM. 2004. Patterns of extrapair mating in relation to male dominance status and female nest placement in Black-capped Chickadees. *Behav. Ecol.* 15:757-765.
- Richardson DS, Burke T. 2001. Extra-pair paternity and variance in reproductive success related to breeding density in Bullock's Orioles. *Anim. Behav.* 62:519-525.
- Rising JD. 2011. Family Emberizidae (buntings and New World sparrows). In: Handbook of the birds of the world, Volume 16 tanagers to New World blackbirds (del Hoyo J, Elliot A, Christie D, eds). Lynx Edicions, Barcelona. Pp 428-683.
- Sánchez JE, Criado J, Sánchez C, Sandoval L. 2009. Costa Rica. In: Important bird areas of Americas: priority sites for biodiversity conservation (Devenish C, Díaz Fernández DF, Clay RP, Davison IJ, Yépez Zabala I, eds). BirdLife International, Quito. Pp 149-156.
- Sclater PL, Salvin O. 1868. Descriptions of new or little-known American birds of families Fringillidae, Oxyrhamphidae, Bucconidae, and Strigidae. *Proc. Zool. Soc. Lond.* 36:322-329.
- Searcy WA, Andersson M. 1986. Sexual selection and evolution of song. *Annu. Rev. Ecol. Syst.* 17:507-533.
- Searcy WA, Anderson RC, Nowicki S. 2006. Bird song as a signal of aggressive intent. *Behav. Ecol. Sociobiol.* 60:234-241.
- Staicer CA, Spector DA, Horn AG (1996) The dawn chorus and other diel patterns in acoustic signaling. In: Ecology and evolution of acoustic communication in birds (Kroodsma DE, Miller EH, eds). Cornell University Press, Ithaca. Pp 426-453.
- Stiles FG, Skutch AF. 1989. Guide to the birds of Costa Rica. Cornell University Press, Ithaca
- Templeton CN, Greene E, Davis K. 2005. Allometry of alarm calls: black-capped chickadees encode information about predator size. *Science* 308:1934-1937.
- Wilson DR, Mennill DJ. 2011. Duty cycle, not signal structure, explains conspecific and heterospecific responses to the calls of black-capped chickadees (*Poecile atricapillus*). *Behav. Ecol.* 22:784-790.

## **Appendix**

**Table S5.1:** Fine-scale song measurements for song type 1 shared between White-eared Ground-sparrow males; coefficients of variation between males (CV<sub>b</sub>) and within males (CV<sub>w</sub>), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. This table accompanies Chapter 5.

Variable	Mean ± SE	CV <sub>b</sub>	CV <sub>w</sub> , mean	PIC	F <sub>1,16</sub>	p
<b>Whole song</b>						
Duration (s)	1.81 ± 0.06	14.90	10.09	1.48	3.48	0.003
Lowest freq. (Hz)	3610 ± 353	33.44	13.93	2.40	3.85	0.001
Highest freq. (Hz)	11392 ± 255	9.92	5.54	1.79	3.28	0.005
Frequency of maximum amplitude (Hz)	6447 ± 84	12.27	10.78	1.14	0.65	0.52
Number of elements	8.86 ± 0.67	27.66	15.22	1.82	3.9	0.001
<b>Middle elements</b>						
Duration (s)	0.34 ± 0.01	15.92	10.32	1.54	3.88	0.001
Lowest freq. (Hz)	4967 ± 279	20.16	5.71	3.53	11.43	<0.001
Highest freq. (Hz)	7587 ± 422	18.35	2.02	9.10	23.91	<0.001
Frequency of maximum amplitude (Hz)	6115 ± 193	12.73	6.73	1.89	4.68	<0.001
Number of inflections	1.92 ± 0.2269	45.57	23.35	1.95	4.61	<0.001
Number of elements	1 ± 0.01	0.00	2.16	0.00	NA	NA
<b>Trill</b>						
Duration (s)	0.98 ± 0.05	19.08	12.99	1.47	2.93	0.01
Lowest freq. (Hz)	3627 ± 363	34.55	13.91	2.48	3.69	0.001
Highest freq. (Hz)	8130 ± 128	6.74	3.46	1.95	2.88	0.01
Frequency of maximum amplitude (Hz)	6984 ± 153	13.85	10.56	1.31	1.28	0.22
Number of inflections	1.55 ± 0.16	41.64	17.07	2.44	3.98	0.001
Number of elements	6.61 ± 0.7	37.62	21.66	1.74	2.73	0.02

**Table S5.2:** Fine-scale song measurements for song type 2 shared between White-eared Ground-sparrow males; coefficients of variation between males (CV<sub>b</sub>) and within males (CV<sub>w</sub>), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. This table accompanies Chapter 5.

Variable	Mean ± SE	CV <sub>b</sub>	CV <sub>w</sub> , mean	PIC	F <sub>1,17</sub>	p
<b>Whole song</b>						
Duration (s)	1.84 ± 0.05	14.25	12.69	1.12	0.88	0.39
Lowest freq. (Hz)	3343 ± 202	25.19	12.19	2.07	4.2	0.001
Highest freq. (Hz)	10604 ± 144	7.24	6.04	1.20	1.52	0.15
Frequency of maximum amplitude (Hz)	6041 ± 217	19.43	15.88	1.22	1.34	0.2
Number of elements	8.05 ± 0.34	22.86	18.25	1.25	1.27	0.22
<b>Middle elements</b>						
Duration (s)	0.23 ± 0.01	23.33	10.46	2.23	6.48	<0.001
Lowest freq. (Hz)	4094 ± 178	17.09	6.53	2.617	4.82	<0.001
Highest freq. (Hz)	6905 ± 314	15.66	4.27	3.67	11.26	<0.001
Frequency of maximum amplitude (Hz)	5203 ± 152	13.65	9.17	1.49	2.5	0.02
Number of inflections	1.03 ± 0.23	85.64	42.61	2.01	2.06	0.06
Number of elements	1.37 ± 0.1	38.88	24.82	1.57	2.33	0.03
<b>Trill</b>						
Duration (s)	1.07 ± 0.04	19.84	15.40	1.29	2.32	0.03
Lowest freq. (Hz)	3481 ± 235	27.18	13.79	1.97	4.17	0.001
Highest freq. (Hz)	8494 ± 177	7.57	2.28	3.32	12.99	<0.001
Frequency of maximum amplitude (Hz)	6825 ± 125	13.70	14.15	0.97	-0.19	0.85
Number of inflections	1.51 ± 0.16	39.38	7.70	5.11	6.16	<0.001
Number of elements	5.31 ± 0.29	30.80	23.53	1.31	1.26	0.23

**Table S5.3:** Fine-scale song measurements for song type 3 shared between White-eared Ground-sparrow males; coefficients of variation between males (CV<sub>b</sub>) and within males (CV<sub>w</sub>), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. This table accompanies Chapter 5.

Variable	Mean ± SE	CV <sub>b</sub>	CV <sub>w</sub> , mean	PIC	F <sub>1,16</sub>	p
<b>Whole song</b>						
Duration (s)	1.88 ± 0.03	10.20	10.49	0.97	-1.16	0.87
Lowest freq. (Hz)	2941 ± 177	26.53	13.78	1.93	5.08	<0.001
Highest freq. (Hz)	10899 ± 228	10.60	8.14	1.30	1.77	0.10
Frequency of maximum amplitude (Hz)	5790 ± 267	20.67	19.67	1.05	0.27	0.79
Number of elements	8.14 ± 0.16	9.22	8.14	1.13	0.56	0.58
<b>Middle elements</b>						
Duration (s)	0.31 ± 0.004	307.86	13.88	22.18	28.25	<0.001
Lowest freq. (Hz)	6203 ± 122	7.15	3.48	2.06	6.85	<0.001
Highest freq. (Hz)	9076 ± 203	7.81	2.51	3.12	12.15	<0.001
Frequency of maximum amplitude (Hz)	7593 ± 200	11.28	7.73	1.46	2.76	0.01
Number of inflections	2.05 ± 0.05	7.76	2.20	3.52	1.55	0.14
Number of elements	2 ± 0	0.00	0.00	0.00	NA	NA
<b>Trill</b>						
Duration (s)	1.08 ± 0.04	17.30	13.73	1.26	1.43	0.17
Lowest freq. (Hz)	2944 ± 168	25.57	13.50	1.89	4.66	<0.001
Highest freq. (Hz)	7233 ± 364	16.00	2.30	6.94	30.02	<0.001
Frequency of maximum amplitude (Hz)	5562 ± 278	20.84	15.01	1.39	1.43	0.17
Number of inflections	2.04 ± 0.1	21.06	10.19	2.07	1.9	0.08
Number of elements	5.17 ± 0.15	14.16	11.79	1.20	0.73	0.47

**Table S5.4:** Fine-scale song measurements for song type 18 shared between White-eared Ground-sparrow males; coefficients of variation between males (CV<sub>b</sub>) and within males (CV<sub>w</sub>), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. Middle element measurements are not presented for Song Type 18 because this song type lacks that part naturally. This table accompanies Chapter 5.

Variable	Mean ± SE	CV <sub>b</sub>	CV <sub>w</sub> mean	PIC	F <sub>1,19</sub>	p
<b>Whole song</b>						
Duration (s)	1.92 ± 0.08	21.67	13.84	1.57	5.91	<0.001
Lowest freq. (Hz)	3513 ± 190	22.76	8.43	2.70	1.86	0.08
Highest freq. (Hz)	11425 ± 173	10.61	7.40	1.43	2.26	0.04
Frequency of maximum amplitude (Hz)	6040 ± 217	19.21	13.07	1.47	1.81	0.09
Number of elements	6.28 ± 0.31	21.48	13.80	1.56	11.55	<0.001
<b>Trill</b>						
Duration (s)	1.39 ± 0.04	141.82	15672.29	0.01	11.62	0.29
Lowest freq. (Hz)	3463 ± 192	22.97	9.18	2.50	5.58	<0.001
Highest freq. (Hz)	8552 ± 420	19.08	3.01	6.35	25.09	<0.001
Frequency of maximum amplitude (Hz)	5915 ± 209	17.98	11.39	1.58	2.23	0.04
Number of inflections	2.07 ± 0.16	34.52	16.76	2.06	3.70	0.002
Number of elements	5.01 ± 0.25	22.76	16.49	1.38	1.16	0.26

**Table S5.5:** Fine-scale song measurements for song type 20 shared between White-eared Ground-sparrow males; coefficients of variation between males (CV<sub>b</sub>) and within males (CV<sub>w</sub>), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. This table accompanies Chapter 5.

Variable	Mean ± SE	CV <sub>b</sub>	CV <sub>w</sub> , mean	PIC	F <sub>1,12</sub>	p
<b>Whole song</b>						
Duration (s)	1.67 ± 0.05	17.77	13.59	1.31	1.17	0.26
Lowest freq. (Hz)	2443 ± 124	15.15	6.81	2.22	4.92	<0.001
Highest freq. (Hz)	11491 ± 268	9.06	5.45	1.66	2.93	0.01
Frequency of maximum amplitude (Hz)	6318 ± 353	22.14	12.93	1.71	1.99	0.07
Number of elements	5.96 ± 0.29	18.23	10.08	1.81	2.14	0.05
<b>Middle elements</b>						
Duration (s)	0.31 ± 0.02	15.12	8.00	1.89	4.67	0.001
Lowest freq. (Hz)	5917 ± 114	5.64	2.27	2.48	7.36	<0.001
Highest freq. (Hz)	8212 ± 97	3.46	1.85	1.87	3.03	0.01
Frequency of maximum amplitude (Hz)	7193 ± 128	8.64	5.86	1.47	2.3	0.04
Number of inflections	2.85 ± 0.07	14.92	10.04	1.49	1.19	0.26
Number of elements	1 ± 0	0.00	0.00	0.00	NA	NA
<b>Trill</b>						
Duration (s)	0.96 ± 0.02	17.22	14.33	1.20	0.78	0.59
Lowest freq. (Hz)	2413 ± 124	15.54	8.04	1.93	4.24	0.001
Highest freq. (Hz)	6632 ± 239	9.19	1.71	5.37	19	<0.001
Frequency of maximum amplitude (Hz)	5745 ± 188	16.14	11.20	1.44	0.95	0.36
Number of inflections	1.94 ± 0.03	14.35	12.71	1.13	0.26	0.8
Number of elements	3.73 ± 0.19	19.64	13.50	1.46	1.09	0.3

**Table S5.6:** Fine-scale song measurements for song type 24 shared between White-eared Ground-sparrow males; coefficients of variation between males (CV<sub>b</sub>) and within males (CV<sub>w</sub>), potential for individual coding (PIC), and results of analysis of variance comparing the coefficients of variation for each song measurement. This table accompanies Chapter 5.

Variable	Mean ± SE	CV <sub>b</sub>	CV <sub>w</sub> , mean	PIC	F <sub>1,13</sub>	p
<b>Whole song</b>						
Duration (s)	1.99 ± 0.12	18.66	10.10	1.85	4.58	0.001
Lowest freq. (Hz)	3930 ± 173	12.63	4.11	3.08	10.14	<0.001
Highest freq. (Hz)	11239 ± 194	6.37	4.69	1.36	2.13	0.05
Frequency of maximum amplitude (Hz)	5937 ± 253	20.56	17.61	1.17	0.97	0.35
Number of elements	7.77 ± 0.53	23.33	13.12	1.78	2.6	0.02
<b>Middle elements</b>						
Duration (s)	0.71 ± 0.07	27.26	4.48	6.08	15.24	<0.001
Lowest freq. (Hz)	3954 ± 148	10.76	3.74	2.87	6.99	<0.001
Highest freq. (Hz)	7764 ± 311	11.09	2.46	4.51	13.94	<0.001
Frequency of maximum amplitude (Hz)	5717 ± 317	18.75	11.96	1.57	2.84	0.01
Number of inflections	2.57 ± 0.3	34.99	21.56	1.62	1.42	0.18
Number of elements	2.54 ± 0.28	30.36	3.16	9.62	11.77	<0.001
<b>Trill</b>						
Duration (s)	0.81 ± 0.09	34.27	15.92	2.15	6.49	<0.001
Lowest freq. (Hz)	4119 ± 186	13.31	5.78	2.30	6.62	<0.001
Highest freq. (Hz)	9408 ± 409	12.32	2.93	4.21	10.38	<0.001
Frequency of maximum amplitude (Hz)	5931 ± 182	19.41	16.66	1.16	0.97	0.35
Number of inflections	1.33 ± 0.23	50.50	13.72	3.68	4.83	<0.001
Number of elements	4.09 ± 0.56	45.49	22.28	2.04	3.17	0.006

Appendix

**Table S6.1.** Playback responses of white-eared ground sparrows (average  $\pm$  SE) separated by the type and the species of playback stimulus, for both the sympatric and allopatric populations. This table accompanies Chapter 6.

	Allopatric				Sympatric			
	Response time (s)	Approach duration (s)	Time (s) inside the speaker area (3m)	Vocal rate (vocalizations per minute)	Response time (s)	Approach duration (s)	Time (s) inside the speaker area (3m)	Vocal rate (vocalizations per minute)
<b>Calls</b>								
Prevost's ground-sparrow	254.75 $\pm$	288.40 $\pm$	3.35 $\pm$	0.60 $\pm$	249.00 $\pm$	284.58 $\pm$	12.83 $\pm$	1.08 $\pm$
	21.24	11.60	3.35	0.23	21.07	11.16	9.03	0.42
White-eared ground-sparrow	235.85 $\pm$	288.85 $\pm$	7.25 $\pm$	1.80 $\pm$	225.21 $\pm$	279.62 $\pm$	9.08 $\pm$	3.83 $\pm$
	25.69	10.01	5.19	0.87	22.49	11.93	6.57	1.44
Large-footed finch	238.80 $\pm$	277.25 $\pm$	6.85 $\pm$	0.40 $\pm$	209.79 $\pm$	273.42 $\pm$	11.79 $\pm$	2.58 $\pm$
	24.72	15.83	5.80	0.13	24.59	15.05	7.66	1.21
Plain wren	250.00 $\pm$	300.00 $\pm$	0.00 $\pm$	1.05 $\pm$	232.21 $\pm$	292.50 $\pm$	4.79 $\pm$	2.42 $\pm$
	23.03	0.00	0.00	0.70	22.47	7.50	4.79	0.80
<b>Songs</b>								
Prevost's ground-sparrow	200.60 $\pm$	275.50 $\pm$	34.65 $\pm$	1.90 $\pm$	183.37 $\pm$	278.29 $\pm$	2.37 $\pm$	2.17 $\pm$
	28.49	16.87	20.98	0.67	24.90	13.09	2.12	0.61
White-eared ground-sparrow	121.35 $\pm$	255.60 $\pm$	34.45 $\pm$	3.30 $\pm$	164.08 $\pm$	224.17 $\pm$	38.92 $\pm$	3.92 $\pm$
	27.49	19.09	16.74	0.85	28.52	21.27	13.79	1.13
Large-footed finch	215.65 $\pm$	284.30 $\pm$	5.95 $\pm$	0.90 $\pm$	242.87 $\pm$	295.50 $\pm$	1.12 $\pm$	5.58 $\pm$
	26.65	12.23	4.69	0.37	20.82	4.50	1.13	4.19
Plain wren	180.20 $\pm$	264.30 $\pm$	15.60 $\pm$	0.75 $\pm$	225.79 $\pm$	290.96 $\pm$	2.00 $\pm$	1.58 $\pm$
	27.89	16.74	9.14	0.26	22.07	9.04	2.00	0.58
<b>Duets</b>								
Prevost's ground-sparrow	109.75 $\pm$	192.90 $\pm$	53.40 $\pm$	4.50 $\pm$	164.96 $\pm$	207.08 $\pm$	37.67 $\pm$	8.12 $\pm$
	26.55	27.83	19.08	0.86	26.61	25.48	14.50	3.81
White-eared ground-sparrow	120.55 $\pm$	196.70 $\pm$	42.50 $\pm$	4.10 $\pm$	83.25 $\pm$	144.42 $\pm$	94.71 $\pm$	4.87 $\pm$
	27.49	26.96	14.63	1.01	23.75	25.79	23.77	1.15
Large-footed finch	194.15 $\pm$	300.00 $\pm$	0.00 $\pm$	2.40 $\pm$	179.25 $\pm$	282.71 $\pm$	1.08 $\pm$	2.75 $\pm$
	27.61	0.00	0.00	0.82	27.56	12.07	1.04	0.69
Plain wren	193.30 $\pm$	289.45 $\pm$	2.60 $\pm$	1.90 $\pm$	204.08 $\pm$	288.92 $\pm$	8.58 $\pm$	3.67 $\pm$
	27.53	10.55	2.60	0.52	23.88	11.08	8.58	0.84

Appendix

**Table S6.2.** Acoustic features of the calls produced by white-eared ground sparrows (average  $\pm$  SE) separated by the type and the species of playback stimulus, for both the sympatric and allopatric populations. This table accompanies Chapter 6.

	Allopatric				Sympatric			
	Minimum frequency (Hz)	Maximum frequency (Hz)	Frequency bandwidth (Hz)	Duration (s)	Minimum frequency (Hz)	Maximum frequency (Hz)	Frequency bandwidth (Hz)	Duration (s)
<b>Calls</b>								
Prevost's ground-sparrow	7571.03 $\pm$ 486.42	11575.27 $\pm$ 718.85	4004.24 $\pm$ 417.32	0.19 $\pm$ 0.056	7982.17 $\pm$ 417.43	11584.73 $\pm$ 647.66	3602.57 $\pm$ 818.54	3602.54 $\pm$ 818.56
White-eared ground-sparrow	7324.22 $\pm$ 343.91	11607.28 $\pm$ 712.68	4283.06 $\pm$ 958.67	0.21 $\pm$ 0.044	7663.48 $\pm$ 552.54	12285.88 $\pm$ 216.36	4622.41 $\pm$ 530.76	4622.41 $\pm$ 530.76
Large-footed finch	7480.55 $\pm$ 0.00	10107.95 $\pm$ 0.00	2627.40 $\pm$ 0.00	0.21 $\pm$ 0.00	7414.02 $\pm$ 225.30	11580.63 $\pm$ 723.98	4166.61 $\pm$ 690.83	4166.60 $\pm$ 690.83
Plain wren	6892.62 $\pm$ 512.19	12792.21 $\pm$ 280.56	5899.59 $\pm$ 282.20	0.23 $\pm$ 0.056	7191.65 $\pm$ 376.91	12424.09 $\pm$ 395.27	5232.43 $\pm$ 537.23	5232.42 $\pm$ 537.24
<b>Duets</b>								
Prevost's ground-sparrow	4171.97 $\pm$ 0.00	11686.00 $\pm$ 0.00	7514.03 $\pm$ 0.00	4.51 $\pm$ 0.00	5564.46 $\pm$ 164.83	11391.05 $\pm$ 286.63	5826.59 $\pm$ 268.95	5826.59 $\pm$ 268.95
White-eared ground-sparrow	5342.08 $\pm$ 263.52	11125.27 $\pm$ 350.61	5783.18 $\pm$ 610.86	4.99 $\pm$ 0.74	5732.58 $\pm$ 226.30	11406.57 $\pm$ 141.15	5673.99 $\pm$ 209.99	5673.99 $\pm$ 209.99
Large-footed finch	4811.72 $\pm$ 381.69	11437.82 $\pm$ 129.01	6626.10 $\pm$ 437.58	4.52 $\pm$ 0.41	5394.29 $\pm$ 260.33	11406.69 $\pm$ 139.41	6012.40 $\pm$ 321.89	6012.43 $\pm$ 321.89
Plain wren	4396.27 $\pm$ 0.00	11334.60 $\pm$ 0.00	6938.33 $\pm$ 0.00	5.40 $\pm$ 0.00	5447.21 $\pm$ 36.64	11338.31 $\pm$ 104.31	5891.10 $\pm$ 117.54	5891.07 $\pm$ 117.56

Appendix

**Table S6.3.** Acoustic features of the calls and duets produced by white-eared ground sparrows (average  $\pm$  SE) separated by the type and the species of playback stimulus, for both the sympatric and allopatric populations. This table accompanies Chapter 6.

	Allopatric				Sympatric			
	Minimum frequency (Hz)	Maximum frequency (Hz)	Frequency bandwidth (Hz)	Duration (s)	Minimum frequency (Hz)	Maximum frequency (Hz)	Frequency bandwidth (Hz)	Duration (s)
<b>Calls</b>								
Prevost's ground-sparrow	7996.87 $\pm$	11339.40 $\pm$	3342.51 $\pm$	0.17 $\pm$	7767.22 $\pm$	12279.36 $\pm$	4512.15 $\pm$	0.24 $\pm$
	250.27	389.28	487.56	0.030	405.85	242.25	540.59	0.050
White-eared ground-sparrow	8056.40 $\pm$	11048.63 $\pm$	2992.24 $\pm$	0.25 $\pm$	8100.69 $\pm$	11857.03 $\pm$	3756.31 $\pm$	0.32 $\pm$
	129.51	102.32	230.74	0.060	366.12	456.43	499.47	0.050
Large-footed finch	6989.09 $\pm$	11839.57 $\pm$	4850.47 $\pm$	0.18 $\pm$	8662.05 $\pm$	11464.46 $\pm$	2802.41 $\pm$	0.32 $\pm$
	290.13	1554.04	1336.68	0.030	311.78	280.17	244.68	0.040
Plain wren	7294.54 $\pm$	10278.42 $\pm$	2983.87 $\pm$	0.20 $\pm$	8785.49 $\pm$	12454.53 $\pm$	3669.04 $\pm$	0.33 $\pm$
	519.61	1266.04	780.24	0.06	541.27	450.13	991.41	0.070
<b>Duets</b>								
Prevost's ground-sparrow	5334.30 $\pm$	11228.80 $\pm$	5894.50 $\pm$	5.24 $\pm$	5305.71 $\pm$	11642.42 $\pm$	6336.73 $\pm$	5.53 $\pm$
	194.02	193.61	302.55	0.38	98.33	71.09	128.53	0.22
White-eared ground-sparrow	5542.34 $\pm$	11645.54 $\pm$	6103.20 $\pm$	5.37 $\pm$	5507.25 $\pm$	11638.33 $\pm$	6131.07 $\pm$	5.22 $\pm$
	221.08	210.10	313.68	0.20	143.46	98.25	192.72	0.34
Large-footed finch	5306.55 $\pm$	11711.82 $\pm$	6405.29 $\pm$	6.82 $\pm$	5378.16 $\pm$	11808.32 $\pm$	6430.15 $\pm$	5.56 $\pm$
	219.01	286.44	393.98	0.42	167.82	120.27	226.35	0.36
Plain wren	5702.47 $\pm$	10788.50 $\pm$	5086.07 $\pm$	5.78 $\pm$	5500.56 $\pm$	11674.56 $\pm$	6173.99 $\pm$	5.15 $\pm$
	145.76	203.16	269.33	1.18	160.06	106.86	183.44	0.37

**Table S7.1.** List of skins used in this study that were measured at Museo de Zoología Universidad de Costa Rica (UCR), Museo Nacional de Costa Rica (MNCR), the Field Museum of Natural History (FMNH), the University of Michigan Museum of Zoology (MZUM), and the Muséum National d'Histoire Naturelle (MNHN).

---

*Melozone biarcuata biarcuata:*

Female: 109482FMNH, 22986FMNH, 109483FMNH, 109480FMNH, 1880-3400MNHN.

Male: 98401MZUM, 108106MZUM, 89016MZUM, 108105MZUM, 212687FMNH, 212685FMNH, 109481FMNH, 23374FMNH, 22988FMNH, 22990FMNH, 22985FMNH, 22987FMNH, 22983FMNH, 22984FMNH, 22989FMNH, 23373FMNH, 212682FMNH.

---

*Melozone biarcuata hartwegi:*

Female: 94608MZUM, 103527MZUM, 103529MZUM, 107783MZUM, 107784MZUM, 1975-798MNHN, 1975-799MNHN, 1975-800MNHN.

Male: 94610MZUM, 94609MZUM, 94607MZUM, 103526MZUM, 103528MZUM, 103530MZUM, 103531MZUM, 107780MZUM, 107781MZUM, 107785MZUM, 103959MZUM, 1975-797MNHN.

---

*Melozone biarcuata cabanisi:*

Female: 3176UCR, 2577UCR, 186MNCR, 6834FMNH, 72939FMNH, 72938FMNH.

Male: 2436UCR, 2435UCR, 1218UCR, 6335, 23050MNCR, 5175MNCR, 23051MNCR, 4561MNCR, 374214FMNH, 6835FMNH, 72940FMNH, 72937FMNH, 1999-2299MNHN, 1999-2297MNHN.

---

**Table S7.2.** List of recordings used in this study that were obtained from Laboratorio de Bioacústica Universidad de Costa Rica (UCR), the Macaulay Library of Natural Sounds Cornell Laboratory of Ornithology (ML), the private collection of Jesse Fagan (JF), and the private collection of Knut Eisermann (KE).

---

*Melozone biarcuata biarcuata:*

15259ML El Salvador, Santa Ana, Cerro Verde; 106025ML El Salvador, Sonsonate, Finca Altamira; KE57 Guatemala, Tukurú, Alta Verapaz, Guaxac; KE74 Guatemala, Solitarius; KE90 Guatemala, Solitarius; JF01 Guatemala, Los Fraijanes; JF02 Guatemala, San Juan La Laguna; JF03 Guatemala, Guatemala City; JF04 Guatemala, Guatemala City; JF05 Guatemala, Guatemala City; JF06 Guatemala, Panajatchel.

---

*Melozone biarcuata cabanisi:*

UCR01066 Costa Rica, Heredia, Calle Hernández; UCR01067 Costa Rica, Heredia, Calle Hernández; UCR01068 Costa Rica, Heredia, Calle Hernández; UCR01069 Costa Rica, Heredia, Calle Hernández; UCR01070 Costa Rica, Heredia, Getsemani; UCR01071 Costa Rica, Heredia, Getsemani; UCR01072 Costa Rica, Heredia, Getsemani; UCR01073 Costa Rica, Heredia, Getsemani; UCR01074 Costa Rica, Heredia, Getsemani; UCR01075 Costa Rica, Heredia, Getsemani; UCR01076 Costa Rica, Heredia, Getsemani; UCR01077 Costa Rica, Cartago, Ujarras; UCR01078 Costa Rica, Cartago, Ujarras; UCR01079 Costa Rica, Cartago, Ujarras; UCR01080 Costa Rica, Turrialba, CATIE; UCR01081 Costa Rica, Heredia, Getsemani; UCR01082 Costa Rica, Heredia, Getsemani; UCR01083 Costa Rica, Heredia, Getsemani; UCR01084 Costa Rica, Heredia, Calle Hernández; UCR01085 Costa Rica, Curridabat, Las Monjas; UCR01086 Costa Rica, Curridabat, Las Monjas; UCR01087 Costa Rica, Curridabat, Las Monjas; UCR01088 Costa Rica, Curridabat, Las Monjas; UCR01089 Costa Rica, Curridabat, Las Monjas; UCR01090 Costa Rica, Curridabat, Las Monjas; UCR01091 Costa Rica, Heredia, Getsemani; UCR01092 Costa Rica, Heredia, Getsemani; UCR01093 Costa Rica, Heredia, Getsemani; UCR01094 Costa Rica, Heredia, Getsemani; UCR01095 Costa Rica, Heredia, Getsemani; UCR01096 Costa Rica, Heredia, Getsemani; UCR01097 Costa Rica, Heredia, Getsemani; UCR01098 Costa Rica, Cartago, Ujarras; UCR01099 Costa Rica, Cartago, Ujarras; UCR01100 Costa Rica, Cartago, Ujarras; UCR01101 Costa Rica, Cartago, Ujarras; UCR01102 Costa Rica, Heredia, Getsemani; UCR01103 Costa Rica, Heredia, Getsemani; UCR01104 Costa Rica, Heredia, Getsemani; UCR01105 Costa Rica, Heredia, Calle Hernández; UCR01106 Costa Rica, Heredia, Calle Hernández; UCR01107 Costa Rica, Heredia, Calle Cienega; UCR01108 Costa Rica, Heredia, Calle Cienega; UCR01109 Costa Rica, San José, Universidad de Costa Rica campus; UCR01110 Costa Rica, San José, Universidad de Costa Rica campus; UCR01111 Costa Rica, Heredia, Getsemani

---

## Vita Auctoris

NAME: Luis Andrés Sandoval Vargas  
PLACE OF BIRTH: San José, Costa Rica  
YEAR OF BIRTH: 1981  
EDUCATION: Escuela de Biología, Universidad de Costa Rica, B.Sc., 2004  
Escuela de Biología, Universidad de Costa Rica, M.Sc., 2008  
Department of Biological Science, University of Windsor, Ph.D., 2014