

FROM GENES TO COMMUNITIES: INTEGRATIVE BIOLOGY OF ACOUSTIC
INTERACTIONS IN NEOTROPICAL SINGING MICE

By

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To Mary and Eugénie--wise and adventurous grandmothers

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Abstract of Dissertation Presented to the Graduate School
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FROM GENES TO COMMUNITIES: INTEGRATIVE BIOLOGY OF ACOUSTIC
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By

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Chair: Steven M. Phelps
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My dissertation addressed proximate and ultimate factors governing acoustic communication in Neotropical singing mice (*Scotinomys*). A major goal of the work was to understand how interspecific communication contributed to the abrupt altitudinal replacement of singing mice in Costa Rica and Panamá. I integrated data from biogeographical surveys, long-term environmental monitoring, reciprocal removal experiments, staged behavioral trials, reciprocal playback experiments, hormonal manipulation, and analysis of neuronal immediate early gene expression. I found that the production of mouse trills is constrained by a performance trade-off between how fast notes are repeated and the frequency bandwidth of each note. Males vary in vocal performance, and females prefer males that are better performers. I also found that vocalizations and associated aggression are modulated by androgens, an important class of steroid hormones that mediate the expression of male secondary sex characters. These data suggest that vocalizations are used to attract mates and repel rivals and provided an important foundation to address the role of song in mediating interspecific interactions. I found that the larger species, *S. xerampelinus*, is behaviorally dominant and excludes *S. teguina* from higher altitudes. Playback

experiments demonstrated that *S. teguina* is silenced and repelled by heterospecific song, whereas *S. xerampelinus* responded to heterospecifics. Thus, response to song reflects underlying dominance and suggests that interspecific communication mediates altitudinal zonation of ecologically similar congeners. To investigate the neural mechanisms involved in species recognition, I studied brains of male *S. teguina* from two populations (allopatric and sympatric with *S. xerampelinus*) that were broadcast heterospecific vocalizations. In contrast to sympatric males, allopatric animals responded to heterospecifics. Both populations exhibited significant induction of *egr-1* in the auditory cortex and medial geniculate in response to conspecifics when compared to control stimuli. However, heterospecific songs elicited stronger responses in sympatry than in allopatry, despite the fact that these songs elicited less singing behavior in sympatry. I suggest that differences in aggressive experience with heterospecifics can modify auditory tuning to match ecologically important stimuli. The integrative nature of the study elucidates links between changes in perception at the neural level and changes in distribution at the geographic level.

CHAPTER 1 INTRODUCTION

Many animals use long-distance acoustic signals to advertise their presence to a network of potential mates and competitors. A rich tradition of studies on acoustic communication in birds, frogs, and insects has led to important insight into disparate disciplines of biology, including ecology and evolution, behavioral endocrinology, and neuroscience (Kroodsma and Miller 1996; Gerhardt and Huber 2002). These advances have necessitated integration of proximate and ultimate factors that govern acoustic signaling. In my dissertation, I aim to synthesize the proximate and ultimate factors mediating acoustic communication in Neotropical singing mice. While features of acoustic communication have been well described in laboratory rodents (Brudzynski 2010), this work attempts to place advertisement signaling in a broader ecological and evolutionary context.

Chapter 2 assesses the role of androgens- an important class of steroid hormones that mediate the expression of male secondary sex characters- in modulating the production of advertisement vocalizations. I manipulated androgen levels in male mice via castration and hormone replacement and measured their aggressive and singing behavior pre- and post-treatment. I discuss the role of androgens in linking gonadal status with decisions about investment in reproductive behaviors.

Chapter 3 explores the influence of androgens on mediating individual variation in male advertisement vocalizations, and the response of females to that variation. Vocalizations from males captured and recorded at different field sites throughout Costa Rica and Panamá were analyzed to assess within- vs. between-individual variation. Vocalizations were then compared to those produced by laboratory animals whose

circulating levels of androgens were experimentally manipulated. The trills were then manipulated and presented to females to determine their response. I discuss the influence of androgens on modulating the production of physically challenging trills, and how signal variation influences female preference.

Chapter 4 investigates the role of acoustic communication in influencing singing mouse geographic limits. I use biogeographical surveys, reciprocal removal experiments, interspecific behavioral trials, and reciprocal playback experiments to determine the biotic and abiotic factors responsible for the abrupt altitudinal replacement of *S. teguina* by *S. xerampelinus*. I discuss how acoustic communication reflects underlying dominance to mediate altitudinal zonation between ecologically similar congeners.

Finally, Chapter 5 investigates the neuronal mechanisms involved in perception of auditory signals from an ecological perspective. Playback experiments were performed on male *S. teguina* sympatric with dominant heterospecifics and in allopatry. I examined song-evoked induction of an immediate early gene in two auditory regions of the brain. I discuss the pattern of character displacement and how agonistic experience with heterospecifics can modify auditory tuning to match ecologically important stimuli.

CHAPTER 2
ANDROGENS MODULATE SONG EFFORT AND AGGRESSION IN NEOTROPICAL
SINGING MICE¹

Background

Androgens are critically involved in the expression and evolution of secondary sex characters (Adkins-Regan 2005), including vocalizations produced in the context of aggression and mate attraction (Ketterson et al. 1996; Ball et al. 2003; Moore et al., 2005; Wilczynski et al. 2005). In birds, frogs, and fish, androgens act on neural pathways and peripheral musculature to influence vocalization rate, signal duration (Bass and Ramage-Healey 2008), amplitude (Connaughton et al. 1997), and dominant frequency (Beani et al. 1995; Cynx et al. 2005). In turn, males that vocalize often and produce longer, louder signals with lower frequencies are often perceived as greater threats by rival males and preferred by females (Klump and Gerhardt 1987; Clayton and Prove 1989; Ryan and Keddy-Hector 1992; Reby et al. 2005; Apicella et al. 2007; Mager et al. 2007; Wyman et al. 2008). Androgens thus play a key role in modulating information content of vocal signals, and their manipulation enables exploration into how hormonal mechanisms influence behavior.

In rodents, androgens modulate the rate of ultrasonic vocalizations (USV) produced in reproductive contexts (Sales 1972; Floody 1981; Nyby et al. 1992; Yamaguchi and Kelly 2002; James et al. 2006; Hammerschmidt et al. 2009). However, examples of androgenic effects on other attributes of mammalian vocalizations are limited to frequency changes observed in human “castratis” of the 17-18th century

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(Jenkins 1998; Nelson 2005) and adult men administered testosterone therapy (King et al. 2001; Akcam et al. 2004; Hartgens and Kuipers 2004). While dominant frequency and amplitude features are correlated with fighting ability and female mate choice in some mammals (Reby et al. 2005; Wyman et al. 2008), the role of androgens in controlling such vocal attributes is poorly known.

Herein, we investigate androgenic effects on vocalizations of Neotropical singing mice (genus *Scotinomys*), diurnal insectivorous rodents distributed throughout Central American cloud forests (Wilson and Reeder 2005). Singing mice provide a tractable mammalian model because adult males commonly emit loud, frequency modulated trills that span wide bandwidths (10-43 kHz; Hooper and Carleton 1976; Miller and Engstrom 2007; Campbell et al. 2010; Figure 2-1; Object 2-1), features characteristic of advertisement signals used to maximize transmission distance and facilitate localization (Bradbury and Vehrencamp 1998). Presentation and subsequent removal of females increases male singing rate (Fernandez-Vargas et al. 2008), and males countersing in response to broadcast songs of intruding males in the field and laboratory (Chapter 4). Thus, singing mouse trills appear to function similarly to frog and bird trills used in male-male aggression and mate attraction (Emerson 2001; Collins 2004).

In this study, we tested the hypothesis that androgens modulate vocal behavior, song characteristics, and aggression in Alston's singing mouse (*S. teguina*) by experimentally manipulating circulating androgen concentrations. We predicted that males receiving no androgen replacement following castration would sing fewer songs and be less aggressive, whereas androgen replacement would maintain or increase song effort and

aggression. Lastly, we examined whether individual differences in androgen titers predicted individual differences in behavior.

Materials and Methods

Study Animals

Experimental animals used in the study were lab-reared offspring (F1) of wild-captured mice from Cerro La Carpintera, Costa Rica. Sexually experienced adult males were assigned randomly to treatment groups and housed individually in cages for 1 month prior to experimentation. Animals were given cat chow and water *ad libitum* and maintained on a 12L:12D cycle at 20 ± 3 °C. The Institutional Animal Care and Use Committee at the University of Florida (UF #200801939) approved all procedures.

Surgical Procedure

Mice were anesthetized with isoflurane (SurgiVet Isotec T³ Classic Isoflurane Vaporizer) and castrated (Cast) bilaterally through a 1 cm scrotal incision. We placed a 10 mm silastic implant (1.47 mm inner diameter X 1.96 mm outer diameter, Dow Corning Corporation, Midland, Michigan) subcutaneously along the dorsal midline. Implants were filled with either 1 mm (15 mg) of testosterone (T; Sigma T1500), 2mm (30 mg) of testosterone, 1mm of dihydrotestosterone (DHT; Sigma A8380), or left empty ($n = 9$ per treatment). The DHT treatment group was included to identify whether androgens were sufficient to cause behavioral and acoustic changes in the absence of aromatization (Nyby et al. 1992). All implants were sealed with silicon adhesive, sterilized with ethylene oxide, and soaked in 0.1% saline solution at 37 °C for 12 hours prior to implantation. Researchers were blind to animal treatment and implant concentrations approximated physiological doses of plasma T from adult field-captured animals (see Results).

Song Recording and Measurement

Fourteen days pre- and post-surgery, we placed mouse cages inside an acoustic chamber (42 x 42 x 39 cm) made from expanded PVC and lined with anechoic foam. Between 0700-1000h, we recorded spontaneous song rate for 30 min, broadcast a single conspecific song, and measured the latency to counter-sing for 5 min. The broadcast song was randomly chosen from among 51 laboratory recordings of 12 wild-caught males. The stimulus was within 2 standard deviations of the population mean for all song parameters (Campbell et al. 2010). We played stimuli at 50 dB SPL at 1 m from a Pioneer TS-250 speaker (flat frequency response 6 - 40 kHz) via an external soundcard (Edirol FA-66). We used an ACO Pacific microphone and preamplifier (Model 7016 and 4116) connected to a laptop running Raven Pro 1.3 via an external soundcard (Edirol FA-66) to record mouse songs at 30 cm. Microphones were calibrated prior to recording sessions with a Brüel and Kjær sound level meter (Type 2219) and calibrator (Type 4230). All songs were sampled at 96 kHz and 24 bits. To analyze recordings, we used automated code written in MATLAB (Phelps, available upon request) to quantify a variety of song parameters including note number, trill rate (note/s), power (dB SPL), frequency bandwidth (kHz), and dominant frequency (kHz) from three spontaneous songs per individual pre- and post-treatment. We then measured a subset of parameters for every note in the trill and used a quadratic function, $f(x) = Ax^2 + Bx + C$, to summarize how parameters changed over the course of a song. For example, to describe frequency bandwidth over the course of the song, $f(x)$ is defined as the bandwidth at note number x , C represents the bandwidth of the first note, B corresponds to change in bandwidth between the first and second notes, and A describes how rapidly bandwidth changes over the course of a song (Figure 2-1D). We

refer to these parameters as note bandwidth A, B, and C in Table 2-1. Finally, we averaged the coefficients of variation ($CV = [s.d./mean] \times 100$) from each song parameter to assess the degree of song variability pre- and post-treatment.

Resident-Intruder Trials

We performed resident-intruder trials to test whether androgens mediate aggressive behavior in *S. teguina*. The day following pre-surgery song recording, we introduced a random novel male (intruder) to the home cage of the treatment animal (resident) and videotaped their interactions for 5 min. Cages of residents were transferred from isolation to the behavior room 30 min prior to initiation of trials. We recorded number of attacks, latency to attack, and number of submissive supine displays of treatment animals. We repeated the same procedure fifteen days post-treatment, but presented treatment animals with novel intruders so that each intruder was used only once.

Testosterone Radioimmunoassay

All animals were euthanized 16 days post-surgery and trunk blood was collected with heparinized capillary tubes. We centrifuged blood at 2500 rcf for 15 min. We used a solid-phase, 96-well plate (Perkin Elmer, Boston, MA, Protein A Flash Plate Plus) radioimmunoassay (RIA) to determine plasma T concentrations as previously described (Hamlin et al., 2011). Briefly, an antibody specific to T (Fitzgerald Industries, Concord, MA, Cat # 20-TR05T) was diluted in phosphate buffered saline gelatin to a concentration of 1:15,000. We incubated plates with antibody for two hours at room temperature. ^3H -labeled steroid was then added at 12,000 counts/min per 100ul and plates were incubated for three hours at room temperature. Prior to sample analyses, we serially diluted a plasma pool to ensure parallelism with the standard curve.

Interassay variance wells were similarly prepared from a plasma pool of 5 animals. Intra-assay variance averaged 3.7%, whereas interassay variance averaged 6.4%. T concentrations of DHT-treated males reflected in part the cross-reactivity of the assay and are reported below but not included in our analyses. Although the T antibody used in our RIA is reported to have 1% cross-reactivity with DHT (Fitzgerald Industries), the "T" levels detected in our DHT-treated castrates suggest androgen cross reactivity is more substantial. It is worth noting, however, that other studies show DHT-treated castrates to have substantial levels of T (e.g. Crews et al. 1978). We cannot rule out the possibility that DHT promotes T release from non-testicular sources. We compared T concentrations of our experimental animals to those of adult males ($n = 12$) sampled in the field and processed in an identical manner.

Data Analysis

We first used analysis of variance (ANOVA) and Kruskal-Wallis tests to assess variation of all pre-treatment measures among groups. Paired- t tests and Wilcoxon signed-rank tests were then used to assess differences within groups pre- and post-treatment. We corrected for multiple comparisons of song parameters using the Benjamini-Hochberg (1995) procedure. ANOVA's were used to compare post-treatment T concentrations and post-pre differences within groups among treatments. We conducted post-hoc comparisons using Tukey's test. To assess the association between circulating concentrations of T and post-measures on song characteristics, we fit a logarithmic curve to individuals across treatments and used linear regressions to highlight patterns within treatment groups. All statistics were performed in JMP, Version 7 (SAS Institute Inc. Cary, NC, 1989-2007). We report means \pm standard error (s.e.) throughout the text unless otherwise noted.

Results

Plasma Androgen Concentrations

T concentrations (\pm s.d.) varied significantly among groups post-treatment (Cast + empty: 0.39 ± 0.1 ng/ml, Cast + DHT: 2.03 ± 3.3 ng/ml, Cast + T (1mm): 5.33 ± 2.8 ng/ml, Cast + T (2mm): 13.28 ± 3.1 ng/ml; $P < 0.0001$) and approximated the physiological range of adult males sampled in the field (0.76 - 6.35 ng/ml).

Resident-Intruder Trials

The number of attacks or latency to attack intruding males did not differ pre- and post-treatment in any group ($P > 0.05$). However, empty-implanted males engaged in more submissive supine displays post-treatment (Pre: 0.1 ± 0.1 , Post: 1.1 ± 0.3 ; $W = 14.0$, $P = 0.01$; Figure 2-2).

Singing Behavior

Pre-treatment measures for all behavioral variables did not differ among groups ($P > 0.05$). Empty-implanted males decreased spontaneous song rate (Pre, 1.6 ± 0.3 songs/10 min; Post, 0.48 ± 0.2 songs/10 min; $W = -22.5$, $P = 0.004$; Fig. 2-3) and increased latency to sing in response to a conspecific song (Pre, 217.6 ± 35 s; Post, 275.3 ± 23 s; $W = 7.5$, $P = 0.03$; Figure 2-3), whereas DHT, T (1mm), and T (2mm)-implanted males increased spontaneous song rate ($W = 9.5, 14.0, 17.5$ respectively, $P < 0.05$) but exhibited no change in latency to respond ($W = -1.5, 1.5, -7.5$ respectively, $P > 0.05$; Figure 2-3).

Song Parameters

Experimental manipulation had significant effects on a variety of song parameters in empty-implanted males, as exemplified in spectrograms of one male's song pre- and post-treatment (Figure 2-4; Objects 2-2 and 2-3). Empty-implanted males

sang songs with fewer notes (Pre, 101.4 ± 6 notes; Post, 54.7 ± 6 notes; $P > 0.001$) that were lower in power (Pre, 71.6 ± 1 dB SPL; Post, 62.6 ± 1.9 dB SPL; $P > 0.001$) with slightly higher dominant frequencies (Pre, 19.9 ± 0.8 ; Post, 22.5 ± 2 kHz; $p = 0.18$; Table 2-1; Figure 2-5). Such changes were manifest in animals starting songs with longer note durations and internote intervals that quickly decreased in power (Note Duration C, INI C, and RMS amplitude B; Table 2-1). Empty-implanted males also sang less stereotyped songs after treatment (Pre, $6.8 \pm 0.3\%$; Post, $11.1 \pm 1.7\%$; $P > 0.05$; Table 2-1; Figure 2-5D). Conversely, implants maintained the majority of song parameters in DHT- and T-treated groups, with the exception of T (1mm)-treated males exhibiting increased frequency bandwidth (Pre, 25.2 ± 0.6 kHz; Post, 26.2 ± 0.5 kHz; $p > 0.01$) and T (2mm)-treated males showing decreased dominant frequency post-treatment (Pre, 21.2 ± 0.7 kHz; Post, 18.8 ± 0.5 kHz; $P > 0.01$; Table 2-1; Figure 2-5). The relationship between circulating T concentrations and number of notes post-treatment showed a highly non-linear effect across treatments ($y = 10.5(\ln)x + 68$, $r^2 = 0.52$, $F_{1,23} = 24.73$, $P < 0.0001$; Figure 2-6), reflected in varying slopes of the relationship within treatment groups. Among empty-implanted individuals, for example, low circulating concentrations (below physiological range of adult males captured in the field) of androgens showed a steep positive correlation with number of notes ($m = 137.6$, $r^2 = 0.63$, $P = 0.02$). T (1mm)-treated males showed a less steep association ($m = 1.5$, $r^2 = 0.11$, $P = 0.4$), whereas T (2mm)-treated males that were above physiological levels of T showed no relationship ($m = -0.71$, $r^2 = 0.05$, $P = 0.56$). We found similar non-linear effects between circulating T concentrations and power ($y = 2.6(\ln)x + 65.8$, $r^2 = 0.57$, $F_{1,23} = 7.53$, $P < 0.0001$), dominant frequency $y = 21.6(\ln)x - 1.1$, $r^2 = 0.26$,

$F_{1,23} = 7.55$, $p = 0.01$, and song variability ($y = -0.017(\ln)x + 0.08$, $r^2 = 0.37$, $F_{1,23} = 11.21$, $P = 0.003$; Figure 2-6B).

Discussion

We explored the role of androgens in modulating singing behavior, song characteristics, and associated aggressive behavior in Alston's singing mouse. We found that androgens influenced a suite of measures, highlighting their role in linking gonadal status to decisions about investment in reproductive behaviors. Some effects of castration were profound and conspicuous (e.g. reductions in song rate, length, and power), whereas their influence on aggression was subtler.

Administration of T and DHT influenced measures of song rate and response latency similarly. Conversely, empty-implanted males sang less and showed longer latencies to respond to conspecific songs. Because DHT cannot be aromatized to estrogen, androgens appear to act directly on androgen receptors to mediate vocal behavior in singing mice. USVs are androgen-dependent in other rodents, though estrogens facilitate the production of vocalizations in rats and gerbils (Floody 1981; Nyby et al. 1992). Androgenic effects on song rate and latency reflect motivational state in many taxa including rodents (Nunez et al. 1978) and likely act on neural pathways that govern vocal production.

Androgens had a profound influence on the structure of vocalizations. When empty-implanted males sang, their trills were shorter, lower in power, higher in frequency, and more variable. Song length is a classic indicator of motivation in anurans and birds (Catchpole et al. 1986; Bensch and Hasselquist 1991; Welch et al. 1998), and power can convey information on receiver intent and condition (Gerhardt and Huber

2002; Forstmeier et al. 2002; Brumm and Todt 2004; Wyman et al. 2008). The fact that empty-implanted males sang at higher frequencies ('castrati effect') while T (2mm) males sang at lower frequencies after treatment indicates that the larynx remains androgen responsive after puberty. Androgens may influence larynx size or modulate expression of myosin heavy chain isoforms that govern the velocity and force of muscular contractions (Fischer et al. 1993; Dabbs and Mallinger 1999), thus providing a potential mechanism for changes in song attributes. The high song variability of empty-implanted males suggest that androgens are involved in coordinating both the fine neuromotor control and highly motivated state associated with song stereotypy (Kao and Brainard 2006; Woolley and Doupe 2008). Thus, the extensive effects of androgens on vocalizations suggests they act on a suite of targets, including brain regions that relay impulses to the larynx and peripheral muscles that influence dominant frequency and amplitude during exhalation (Beani et al. 1995).

While androgens had a profound influence on song, their effects on aggression were significant but subtler. Androgens significantly reduced the probability of submissive supine behavior, but we found relatively little offensive aggression in any treatment group. Moreover, animals exhibited low levels of offensive aggression prior to castration, indicating that the surgery itself was not causing low aggression levels. We suspect that our testing procedures contributed to low levels of aggression. Blondel (2004) placed male residents of *S. xerampelinus*, a sister species, in the testing room the day before testing, performed trials under red light, and found that animals were very aggressive. The fact that we staged encounters 30 min after placing males in the novel testing room may have led subjects to behave as intruders rather than residents.

Nevertheless, the ability of castration to promote supine displays indicates androgens modulate aggression as well as vocalization in singing mice.

Interestingly, the influence of androgens on vocal attributes followed a strongly non-linear relationship (Figure 2-6). The relationship between number of notes and plasma testosterone titers, for example, is steepest in the range of hormone concentrations exhibited by castrates, shallow in the range of intact males, and flat at supra-physiological levels. We interpret such a curve as a type of “reaction norm”, which describes how animals translate variation in circumstance (in this case reproductive status signaled by androgen concentrations) into changes in phenotype (vocal effort). If this relationship is adaptive, the steep increase in vocal effort elicited by T concentrations just below those of healthy adult males may be a means of ensuring that males engage in high vocal effort when testes are producing gametes, or none at all when conditions are unfavorable for reproduction. The more shallow relationship within the range of adult T titers suggests that natural variation in androgens coordinate subtle differences in vocal effort among males. Lastly, the flat relationship at supra-physiological doses suggests that increasing vocal effort beyond that plateau will provide no further increase in fitness regardless of reproductive condition or social status. Such non-linear curves were consistent across measures of vocalization, and have long been observed in behavioral endocrinology (Beach 1948; Adkins-Regan 2005; Ball and Balthazart 2008). Reaction norms could provide a useful and general framework for investigating the relationship between hormone concentrations and behavior.

The hormonal coordination of multiple dimensions of vocal effort is similar to proposed endocrine control over vocalization rates in anurans (Marler and Ryan 1996; Emerson 2001; Leary et al. 2004). The model suggests that androgens have graded effects on vocal characteristics and are suppressed by negative feedback from corticosterone associated with energetic constraints of calling. Future studies that incorporate androgenic interactions with other hormones and neuromodulators will provide important insight into the full suite of mechanisms involved in signal production. By introducing a novel mammalian species, we hope to broaden the taxonomic scope of work in acoustic signaling to facilitate integration of hormones, behavior, and evolution.

Table 2-1. Results of paired-*t* tests of acoustic variables for male singing mice post - pre treatment. Bolded values are significant after Benjamini-Hochberg procedure (Q = 0.10). INI = inter-note interval, RMS amp = root mean square amplitude, DF = dominant frequency. ^a Change is RMS Amp is linear (values = 0).

Acoustic variable	Empty		DHT (1mm)		T (1mm)		T (2mm)	
	<i>t</i> ₈	<i>p</i>	<i>t</i> ₈	<i>p</i>	<i>t</i> ₈	<i>p</i>	<i>t</i> ₈	<i>p</i>
<u>Amplitude measures</u>								
# notes	-6.42	0.0002	0.13	0.89	-0.37	0.72	-0.58	0.57
Trill rate (notes/s)	-1.16	0.27	-2.03	0.07	-2.01	0.07	-0.23	0.82
Power	-5.34	0.0007	1.76	0.11	0.78	0.45	0.23	0.82
Note duration A	-1.51	0.16	0.88	0.41	2.06	0.07	-0.04	0.96
Note duration B	2.51	0.03	0.19	0.85	-0.77	0.46	0.67	0.51
Note duration C	1.37	0.02	-0.24	0.81	2.10	0.06	-0.15	0.88
INI A	2.02	0.07	1.38	0.20	2.82	0.02	1.13	0.28
INI B	0.66	0.52	-1.12	0.29	-2.92	0.01	-1.21	0.25
INI C	2.59	0.03	1.16	0.27	1.50	0.17	2.03	0.07
Note RMS Amp A ^a	-	-	-	-	-	-	-	-
Note RMS Amp B	-3.32	0.01	1.28	0.23	0.15	0.88	0.70	0.49
Note RMS Amp C	0.63	0.54	-0.55	0.59	-0.47	0.65	-1.74	0.11
<u>Frequency measures</u>								
Bandwidth	-1.19	0.08	1.05	0.32	3.48	0.008	-0.27	0.79
Dominant frequency	1.46	0.18	0.74	0.47	-1.29	0.23	-3.72	0.005
Note bandwidth A	0.15	0.88	0.46	0.65	-0.28	0.78	-0.71	0.49
Note bandwidth B	0.28	0.78	-0.32	0.75	0.07	0.94	0.40	0.69
Note bandwidth C	0.07	0.93	0.66	0.52	1.15	0.28	-0.21	0.83
Note DF A	0.61	0.55	-0.63	0.54	-0.06	0.94	0.37	0.71
Note DF B	0.13	0.89	1.30	0.22	-0.25	0.80	-1.12	0.29
Note DF C	0.38	0.71	-1.40	0.19	0.58	0.57	2.51	0.03
<u>Stereotypy measure</u>								
Song variability	2.77	0.02	-0.50	0.62	-1.45	0.18	-0.49	0.64

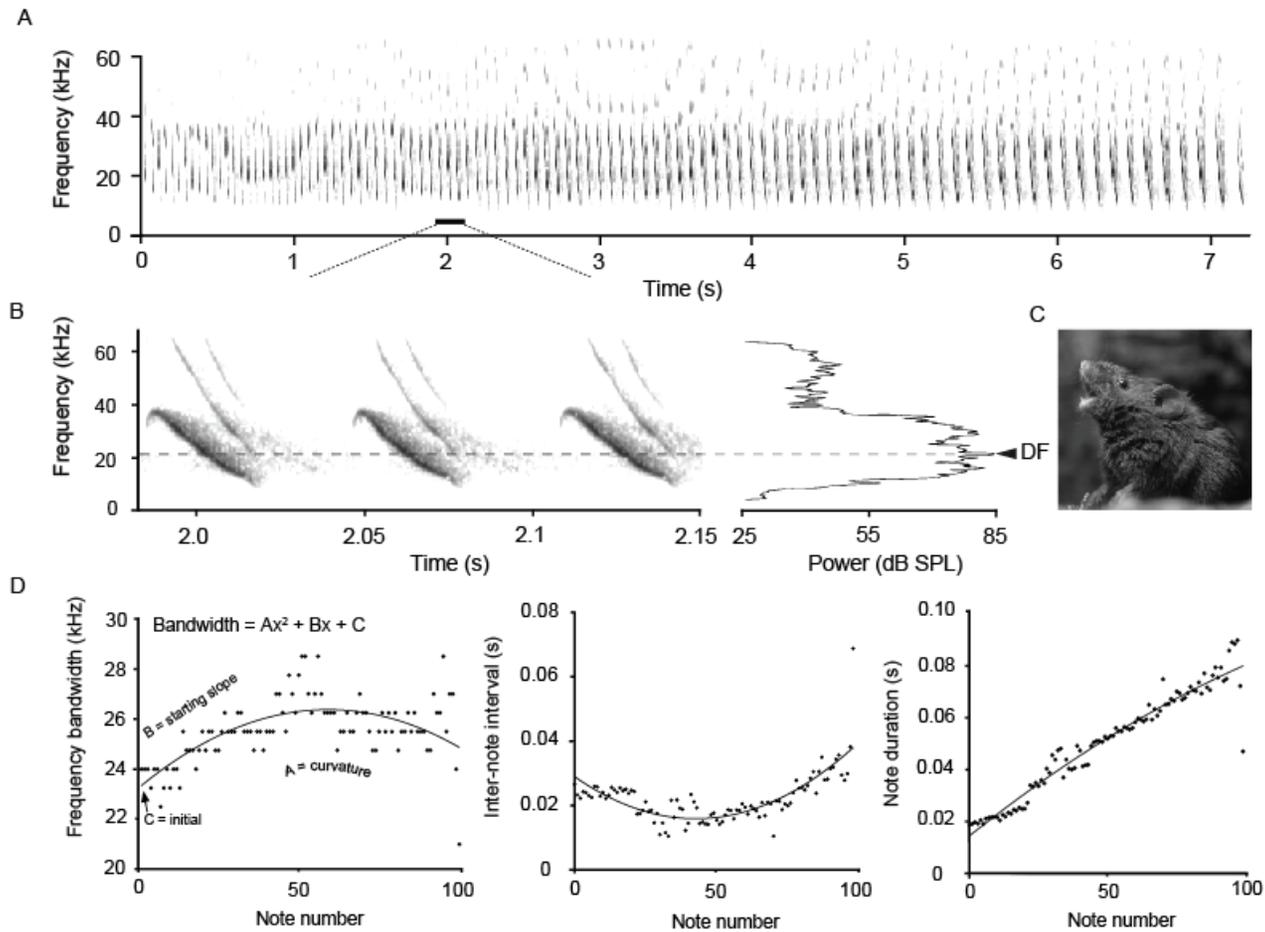


Figure 2-1. The song of Alston's singing mouse (*S. teguina*). A) Spectrogram of Alston's singing mouse (*S. teguina*) song. B) Spectrogram and power spectrum of 3 notes underlined in A). C) Adult male *S. teguina* singing. (D) Note measurements on change in frequency bandwidth, inter-note interval, and note duration over the course of song. DF = dominant frequency.

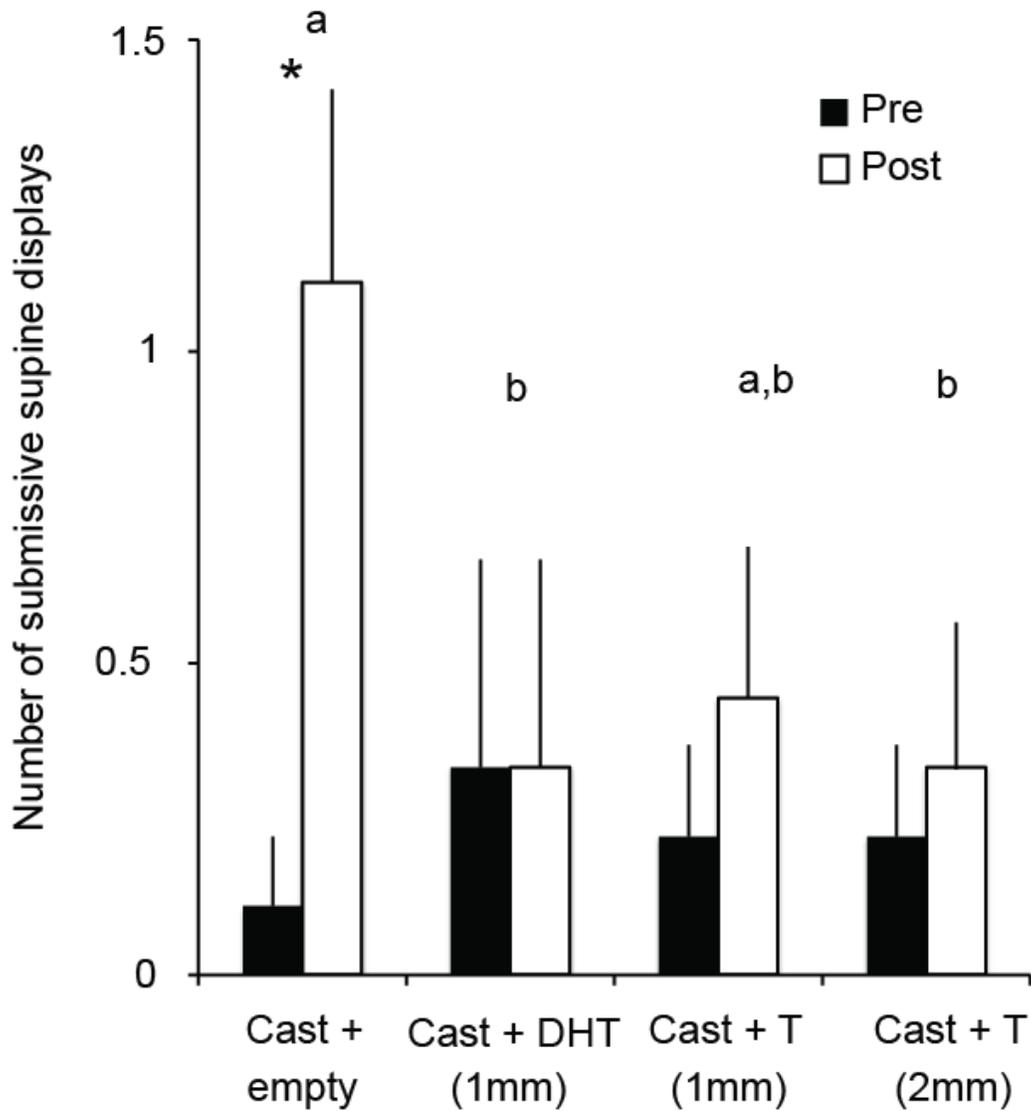


Figure 2-2. Androgenic effects on aggressive behavior. Number of submissive supine displays by resident male singing mice to intruding male conspecifics pre- and post-treatment. Error bars are + 1 s.e. * $p < 0.05$ for post – pre differences within groups. Groups not sharing the same letter have significantly different post – pre values (Tukey HSD, $p < 0.05$). Cast= castrate, DHT = dihydrotestosterone, T = testosterone.

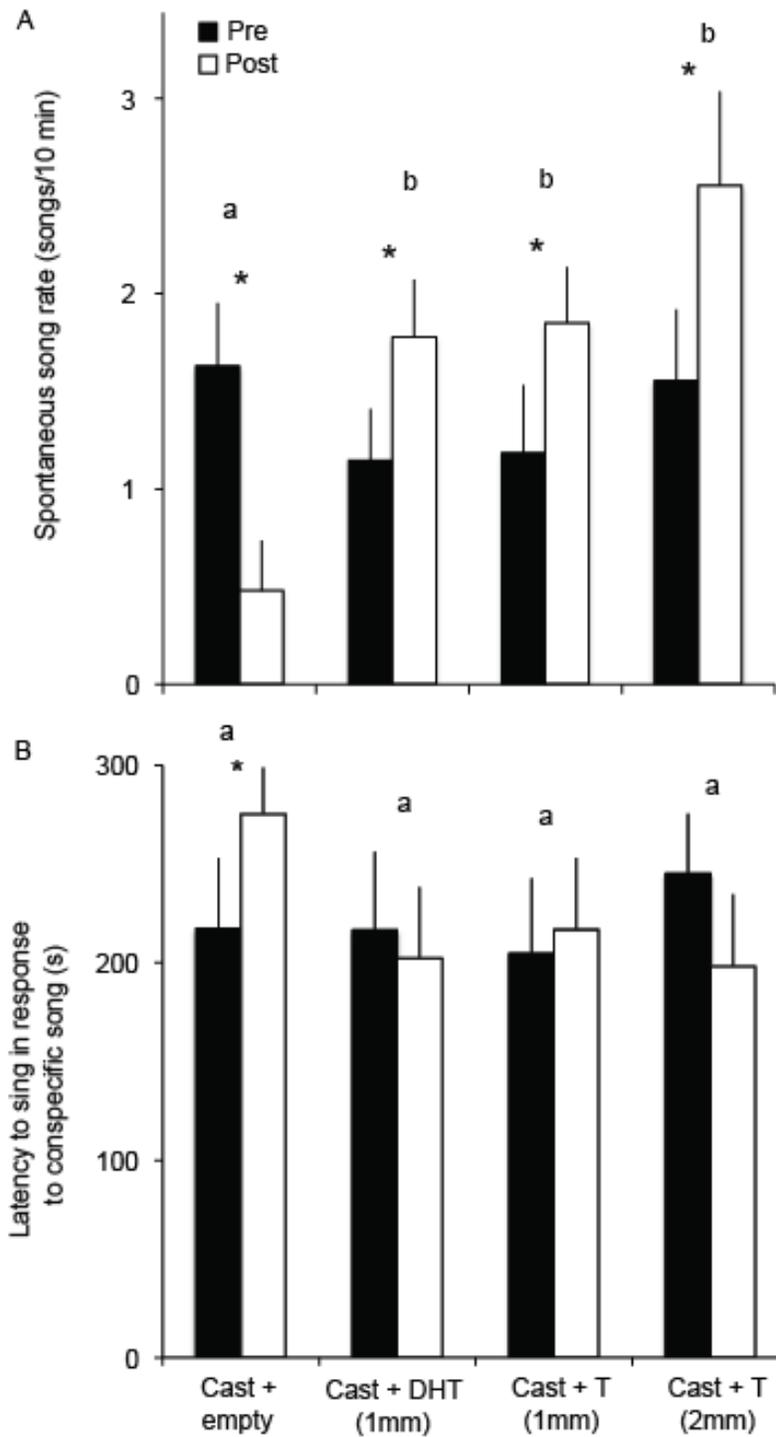


Figure 2-3. Androgenic effects on singing behavior. A) Spontaneous song rate (songs/10 min) and B) latency to sing (s) in response to conspecific song of male singing mice pre- and post-treatment. Error bars are + 1 s.e. * $p < 0.05$ for post – pre differences within groups. Groups not sharing the same letter have significantly different post – pre values (Tukey HSD, $p < 0.05$). Cast = castrate, DHT = dihydrotestosterone, T = testosterone.

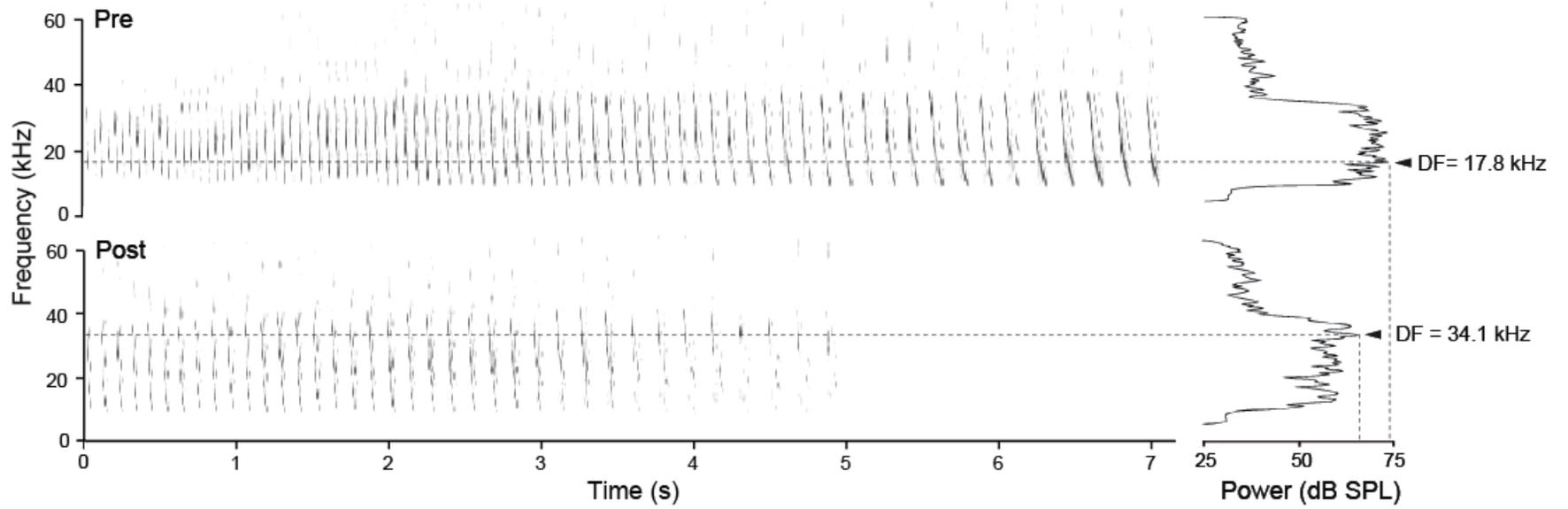


Figure 2-4. Mouse song pre- and post- castration. Representative songs of an individual male singing mouse castrated + empty-implant pre- and post treatment. DF= dominant frequency.

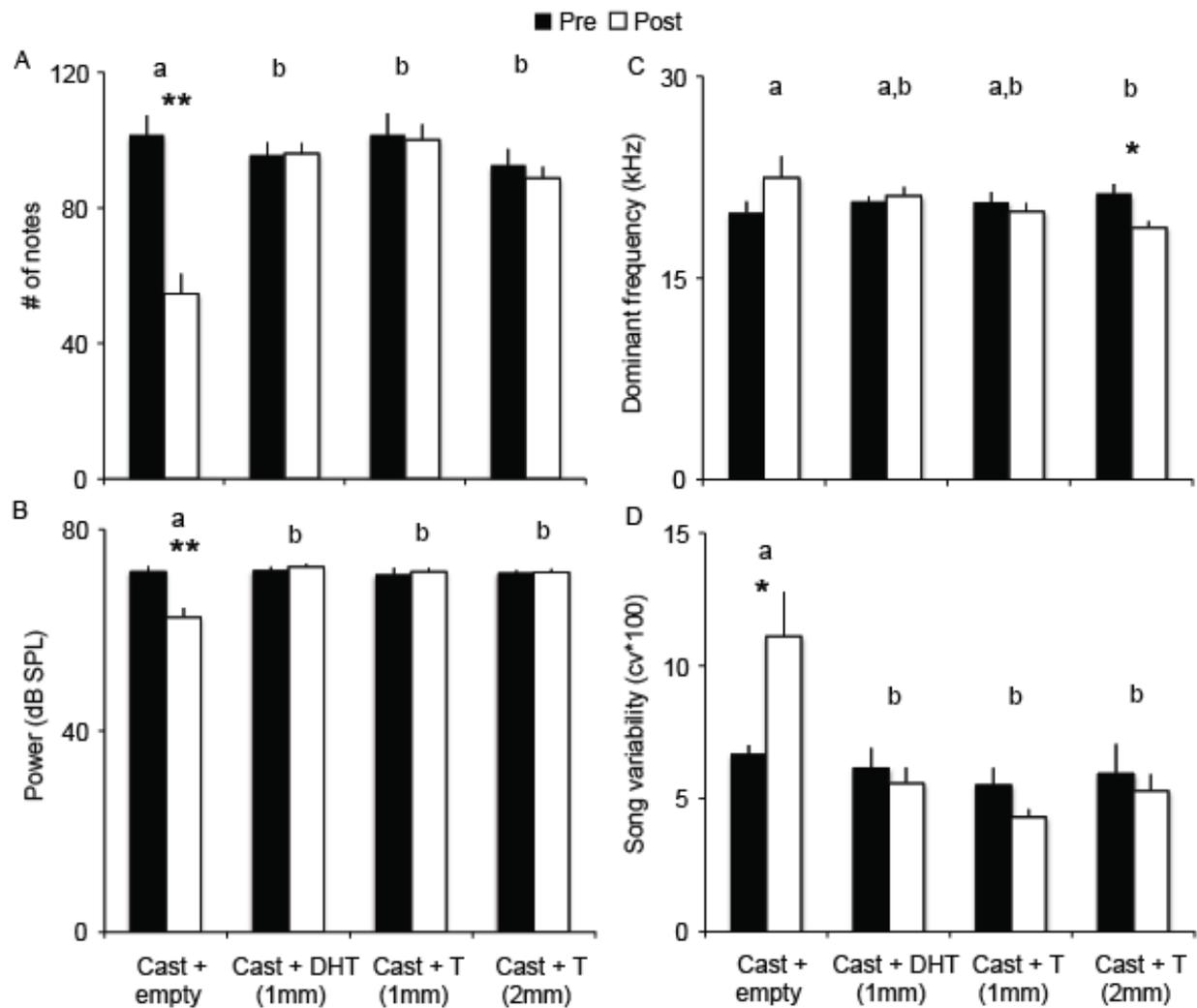


Figure 2-5. Androgenic effects on song parameters. A) Number of notes, B) Power (dB SPL at 30 cm, C) Dominant frequency (kHz), and D) Song variability (CV *100) of male singing mice pre- and post- treatment. Error bars are + 1 s.e. ** $p < 0.001$, * $p < 0.05$ for post - pre differences within groups. Groups not connected by same letter have significantly different post - pre values (Tukey HSD, $p < 0.05$). Cast= castrate, DHT = dihydrotestosterone, T = testosterone.

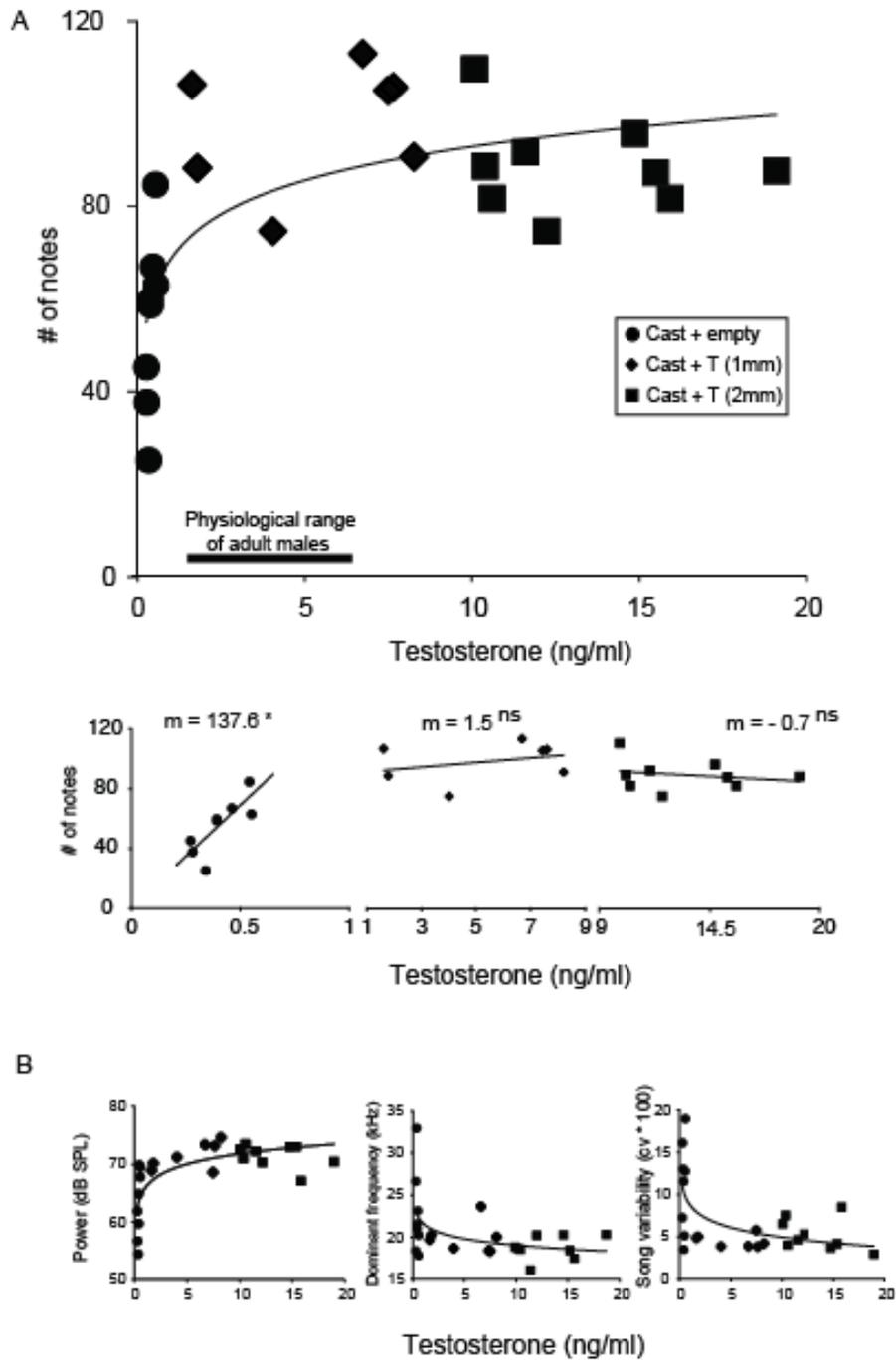


Figure 2-6. Relationship between androgen concentrations and song parameters in singing mice. A) Testosterone concentrations (ng/ml) of hormone-treated male singing mice by number of notes. Black line along x-axis indicates the range of physiological concentrations of testosterone from field-captured mice in Costa Rica and Panamá. m = slope, * $p < 0.10$. B) Testosterone concentrations (ng/ml) of hormone-treated male *S. teguina* by power (dB SPL at 30 cm), dominant frequency (kHz), and song variability (CV * 100).

Object 2-1. Song of intact male Alston's singing mouse recorded at 96 kHz. (.wav file 0.71 MB)

Object 2-2. Song of male Alston's singing mouse prior to castration. Recorded at 96 kHz and played back at ½ speed. (.wav file 1.82 MB)

Object 2-3. Song of male Alston's singing mouse after castration. Recorded at 96 kHz and played back at ½ speed. (.wav file 0.91 MB)

CHAPTER 3
ANDROGEN-DEPENDENT MALE VOCAL PERFORMANCE INFLUENCES FEMALE
PREFERENCE IN NEOTROPICAL SINGING MICE¹

Background

Vocalizations used in aggressive and mating contexts are among the most diverse and elaborate displays in the animal kingdom. Physical or physiological constraints can result in signals that convey information about signaler condition (Maynard Smith and Harper 1995; Vehrencamp 2000; Fitch and Hauser 2003; Hurd and Enquist 2005; Taylor and Reby 2010). Receiver responses to such signals can generate substantial intra- and inter-sexual selection (Andersson and Simmons 2006). In birds, anurans, and mammals, conspicuous features of vocal signals (e.g. duration and fundamental frequency) provide accurate indicators of male quality and size that are attractive to females and threatening to rival males (Clayton and Prove 1989; Welch et al. 1998; Reby et al. 2005). Such displays are thought to transmit honest information because only individuals in better condition are able to afford costs associated with signal production.

In vertebrates, vocal displays associated with reproduction are often mediated by androgens released from the testes (Floody 1981; Ball et al. 2003; Moore et al. 2005; Bass and Remage-Healey 2008). Androgens can influence vocalizations through actions on motivational centers and vocal motor pathways in the central nervous system (reviewed in Yamaguchi and Kelly 2002; Bass and Remage-Healey 2008) or via modulation of peripheral structures involved in signal production. For example, changes

¹ Reprinted with permission from *Animal Behaviour*.

Pasch, B., George, A.S., Campbell, P., and S.M. Phelps. 2011a. Androgen-dependent male vocal performance influences female preference in Neotropical singing mice. *Animal Behaviour* 82:177-183.

in fundamental frequency result from anabolic effects of androgens on the larynx and syrinx, the 'source' of sound production in birds and mammals (Beckford et al. 1985; Beani et al. 1995; Cynx et al. 2005; Evans et al. 2008; Pasch et al. 2011; Taylor and Reby 2010). By modulating signal duration, rate, or fundamental frequency, androgens can provide a mechanistic link between male condition and signal form (Remage-Healey and Bass 2007; Charlton et al. 2011).

While androgens influence conspicuous features of vocal signals, little is known about their effects on vocal attributes that reflect male performance. For example, many avian species emit a series of rapidly repeated notes of similar structure termed trills. Trill production is constrained by a mechanical trade-off between how fast notes are repeated (trill rate) and the frequency bandwidth of each note, resulting in a performance limit (Podos 1997). Males with high vocal performance scores are considered greater threats by rival males and are more attractive to females in a variety of species (Ballentine et al. 2004; Illes et al. 2006). Vocal performance is also correlated with age, social status, and reproductive success (Janicke et al. 2008; Ballentine 2009; Botero et al. 2009; de Kort et al. 2009). Despite the association between circulating testosterone and these same variables (age: Morton et al. 1990; Vleck and Brown 1999, social status: Wingfield 1985; Wingfield and Moore 1987, reproductive success: Reed et al. 2006), the role of androgens in mediating variation in vocal performance is unclear.

In birds, vocal performance trade-offs are thought to arise via 'filter' constraints whereby sound frequencies track beak gape, with wide gapes corresponding to high frequencies and small gapes corresponding to low frequencies (Westneat et al. 1993; Hoese et al 2000). However, other aspects of respiratory and syringeal motor patterns

are important in modulating aspects of note rate and frequency modulation (Goller and Suthers 1996; Nelson et al. 2005; Riede et al. 2006). In particular, 'minibreaths' that birds take between each note suggest a potential role for respiratory musculature in limiting temporal aspects of note production (Calder 1970; Hartley and Suthers 1989; Lambrechts 1996). While the influence of androgens on avian respiratory musculature has not been well characterized, the mammalian diaphragm is highly sensitive to androgens that can influence the velocity and force of respiration (Prezant et al. 1997). Similarly, the craniomandibular musculature of rodents is responsive to androgens (Lyons et al. 1986; Eason et al. 2000), which suggests a mechanism by which androgens could influence the rate of gape and frequency modulation. These data suggest that androgens could provide an important yet underappreciated mechanism modulating vocal performance, and highlight the potential for an appropriate mammalian model to inform our understanding of vocal signals.

Neotropical singing mice (genus *Scotinomys*) are diurnal insectivorous rodents that inhabit montane cloud forests throughout Central America (Hooper and Carleton 1976; Wilson and Reeder 2005). Adult males commonly emit stereotyped trills that are androgen-dependent and used in male-male aggression and mate attraction (Pasch et al. 2011; herein). Presentation and subsequent removal of females increases male song rate (Fernandez-Vargas 2006), and males countersing in response to broadcast songs of intruding males in the field and laboratory (Pasch et al. 2011; Chapter 4). When singing mice trill (Object 2-1), they take 'minibreaths' between each note and appear to modulate the fundamental frequency (10-43 kHz) with their mouth gape (Miller and

Engstrom 2007; Object 3-1) in a manner analogous to some bird song (Suthers et al. 1999).

In this study, we first ask if Alston's singing mouse (*S. teguina*) exhibits a vocal performance trade-off between trill rate and frequency bandwidth. We then manipulate androgens in lab-reared males to investigate whether they play a role in modulating vocal performance. Finally, we experimentally manipulate trills to determine if females responded to versions resembling those of androgen-treated males. We predicted that androgen removal would negatively impact vocal performance, and that females would prefer male mouse trills with higher performance scores over those with lower scores.

Materials and Methods

Generating the Population Performance Limit

To assess variation in male trills, we live-captured animals in Sherman traps baited with peanut butter and oats in four localities throughout Costa Rica and Panamá from 2006-2009 (see Campbell et al. 2010). We measured their mass and hindfoot length prior to transferring males to PVC-coated wire mesh cages (28 cm x 28 cm x 28 cm). Cages were placed inside anechoic recording chambers (42 cm x 42 cm x 39 cm) made from expanded PVC. Details on song recording can be found in Campbell et al. (2010). We recorded spontaneous trills from 102 males ($N = 3.73 \pm 1.57$ trills/male, range 1-12). Animals were uniquely marked and released at the site of capture. All capture and recording procedures in the field were conducted with approval from the Institutional Animal Care and Use Committee (IACUC) at the University of Florida (#E436), the Costa Rican Ministerio del Ambiente y Energia, and the Panamanian Autoridad Nacional del Ambiente.

We used an automated code in MATLAB (available upon request) to quantify the average trill rate and frequency bandwidth for the entire song. Frequency bandwidths were calculated by a threshold routine that identified frequencies at the onset and offset of notes that were 8 standard deviations above the average background noise (sampling rate: 96 kHz; Fast Fourier Transform size: 256; Hann window, time resolution = 50 ms). We plotted an average of each male's songs (filled circles, Figure 3-2A) and calculated vocal performance limits as described in detail elsewhere (Podos 1997; Ballentine et al. 2004). Briefly, we first pooled each male's average trill into 1 note/s bins. For each bin, we determined the male with the largest frequency bandwidth (open circles, Figure 3-2A) and calculated a linear regression through these maxima (after Blackburn et al. 1992). This statistical technique attempts to describe biological boundaries by defining theoretical limits to bivariate plots. In this context, the upper bound regression operationally defines the population performance limit on trill production, with trills closer to the limit being more difficult to produce (Podos 1997).

Androgen Manipulation

We experimentally manipulated androgen levels of lab-reared offspring (F1) of wild-captured mice from Cerros de la Carpintera, Costa Rica. Sexually experienced adult males were assigned randomly to one of three treatment groups and housed individually in cages for 1 month prior to experimentation. Animals were given cat chow and water *ad libitum* and maintained on a 12L:12D cycle at 20 ± 3 °C. Mice were anesthetized with isoflurane (SurgiVet Isotec T³ Classic Isoflurane Vaporizer) and castrated (Cast) bilaterally. We placed a 10 mm silastic implant (1.47 mm i.d. X 1.96 mm o.d., Dow Corning Corporation, Midland, Michigan) subcutaneously along the

dorsal midline. Implants were filled with either 1 mm (15 mg) of testosterone (T; Sigma T1500), 1 mm of dihydrotestosterone (DHT; Sigma A8380), or left empty ($N = 9$ per treatment). The DHT treatment group was included to identify whether androgens were sufficient to cause acoustic changes in the absence of aromatization (Nyby et al. 1992). Implants were sealed with silicon adhesive, sterilized with ethylene oxide, and soaked in 0.1% saline solution at 37 °C for 12 hours prior to implantation. Implant concentrations approximated physiological doses of plasma T of field-captured animals (see Pasch et al. 2011).

Fourteen days pre- and post-surgery, we placed mouse cages inside an anechoic recording chamber to record trills ($N = 3/\text{male}$) as described above. We then plotted trill rate and frequency bandwidth for all experimental animals pre- and post-treatment and calculated performance scores as the orthogonal deviation (i.e. vocal deviation in Podos 2001) from the performance limit generated from field-captured males. Researchers were blind to animal treatment. All laboratory procedures were conducted with permits from IACUC at the University of Florida (#200801939).

Female Phonotaxis

To assess female response to male trills, we live-captured females at Cerros de la Carpintera, Costa Rica in February 2010. We used only parous females ($N = 18$) as indicated by open pubic symphyses and enlarged nipples, because pregnant and lactating females ($N = 4$) showed no phonotaxis towards male trills in preliminary studies. We isolated females in wire mesh cages placed inside anechoic chambers for 48 hours prior to experimentation. Females were given cat chow and water *ad libitum* and were released at the site of capture after our experiments.

To synthesize experimentally high performance scores, we randomly selected 6 trills recorded from different males from the same population (“Slow”; S) and deleted 70% of gaps of silence between each note using bioacoustics software Raven Pro 1.3 (Cornell Lab of Ornithology, Ithaca, NY). Thus for each Slow stimulus we had a corresponding synthetic (“Fast”; F) stimulus that differed only in its trill rate and duration. By increasing trill rate without changing bandwidth, Fast trills were consistently closer to the population performance limit (Slow: -6.8 ± 0.90 ; Fast: -1.6 ± 0.92 ; Paired t test, $t_5 = 12.16$, $P < 0.0001$; Figure 3-3A). Each Slow-Fast stimulus pair was used to test 3 different females. Thus we used a total of 6 stimulus pairs to test 18 females.

We tested females in a 92 cm x 8 cm x 23 cm arena made from translucent white acrylic with a central compartment to house the female subject. The arena was a single runway 8 cm wide, 23 cm tall, and 92 cm long, bisected by a central compartment 9 cm x 8 cm for housing the subject female. Each sliding door of the central chamber had a 5 cm diameter hole covered with stainless steel mesh to allow females to hear stimuli. Two speakers (Pioneer TS-250; flat frequency response 6 - 40 kHz) were mounted at opposite ends (Figure 3-1A). Trills were broadcast from a laptop to each speaker via an external soundcard (Edirol FA-66) with a peak amplitude of 50 dB SPL at 1 m, which approximates natural trill amplitudes. Speakers were calibrated daily with a Brüel and Kjær sound level meter (Type 2219) and calibrator (Type 4230). Females were placed in the central chamber of the choice arena between 1500-1830 two days following capture. We allowed females to acclimate for 17 min prior to the first test stimulus. Slow and Fast stimuli from the same male were broadcast antiphonally from each speaker beginning 3 min before the doors were lifted. Each stimulus was repeated every 60 s,

and the onset of the two stimuli were 30 s out phase. We then ceased playback, opened the doors and recorded female responses for 5 min. After each trial, the arena was cleaned with water and alcohol and the female was placed in the central chamber for a 17 min inter-trial acclimation. The same stimuli were used for a second test, but stimulus order and side were reversed. We calculated the latency to approach (300 s if no approach) and time spent near (within 30 cm of speaker) each stimulus.

Researchers blind to treatments scored all trials.

Statistical Analyses

Linear regressions were used to determine the relationship between trill rate and frequency bandwidth, to calculate the performance limit, and to assess how body condition influenced performance scores of male trills recorded in the field. We used model II analysis of variance (ANOVA) to test whether variation in vocal performance was greater among vs. within individuals. Variance components were then used to estimate repeatability of vocal performance within males (Lessells and Boag 1987). For experimentally manipulated animals in the lab, we used ANOVA to assess variation of pre- and post-treatment performance scores among groups, and paired *t* tests to assess differences within groups pre- and post-treatment. Significance of post-treatment performance scores among groups was assessed using Bonferroni-corrected alpha values ($\alpha = 0.02$).

To estimate female response to male vocal performance, we identified the speaker that was approached first following playback. The null hypothesis of no preference predicts females will be equally likely to approach either stimulus in a given test. Expanding that to a two-test paradigm (e.g. Kime et al. 1998), we predicted that 25% of females would initially approach the Fast stimulus twice ("FF", $0.5 \times 0.5 = 0.25$), 25%

would initially approach the Slow stimulus twice (“SS”), and 50% would first approach one stimulus in each test (“FS”, $0.5*0.5 + 0.5*0.5 = 0.5$). We used a chi-square test to determine deviations from these expectations. To further assess female responses, we calculated the difference in time spent within 30 cm of the speaker broadcasting each stimulus, averaged across the two trials and within each stimulus type, and then performed a paired *t*-test to ask whether females spent more time with Fast or Slow stimuli. We used the same analysis to examine the latency to approach each speaker. Positive values from paired *t* tests indicated that females showed a preference for Fast stimuli, negative values indicated that females showed a preference for Slow stimuli, and 0 indicated no preference for either stimulus (either moving back and forth to each stimulus or exhibiting place preference after the first trial). In a similar manner, we tested for order effects by assessing differences in approach latencies and time spent near each speaker within and among trials. Lastly, we examined the relationship between female responses to the Fast stimulus and its performance score. We averaged a female’s latency to approach the Fast stimulus across the two trials and within each stimulus type and regressed this average against the performance score for the corresponding stimulus. Similarly, we regressed the average time spent near the speaker broadcasting the Fast stimulus against the Fast stimulus’ performance score. All statistical analyses were performed in SAS Version 9.2 and JMP, Version 7 (SAS Institute Inc., Cary, NC, 1989-2008). We report means \pm standard errors (SE) throughout the text unless otherwise noted.

Results

Population Performance Limit

We found a negative relationship between trill rate and frequency bandwidth from Costa Rican and Panamanian field populations ($y = -466.8x + 30080$, $R^2 = 0.10$, $F_{1,101} = 11.45$, $P = 0.001$; Figure 3-2A). Regression of maximum bandwidth against trill rate revealed a negative relationship, operationally defined as the performance limit ($y = -1191.4x + 42692$, $R^2 = 0.72$, $F_{1,8} = 20.12$, $P = 0.002$). We found greater among- vs. within-individual variation in vocal performance ($F_{101,280} = 4.80$, $P < 0.0001$), which showed a moderate level of repeatability (0.52). Body condition showed a significant but weak positive association with performance scores of field-captured animals ($R^2 = 0.05$, $F_{1,96} = 5.01$, $P = 0.027$).

Androgenic Effects on Performance Scores

We found no significant group differences in pre-surgery performance scores as expected given random assignment of treatments (ANOVA: $F_{2,24} = 1.71$, $P = 0.20$). However, performance scores of empty-implanted males fell significantly away from the population performance limit following castration (Paired t test: $t_8 = -3.01$, $P = 0.01$; Figure 3-2B). Conversely, performance scores of DHT and T-treated animals remained at pre-castration levels (DHT: $t_8 = -0.49$, $P = 0.63$; T: $t_8 = 0.47$, $P = 0.64$). The influence of androgens was reflected in a significant treatment effect on post-castration performance (ANOVA: $F_{2,24} = 3.89$, $P = 0.03$). Both T- and DHT- treated animals differed marginally from empty-implanted controls (T vs. Empty, $t_{17} = 3.27$, $P = 0.09$; DHT vs. Empty, $t_{17} = 6.72$, $P = 0.02$), but did not differ from one another ($t_{17} = 1.05$, $P = 0.34$).

Female Phonotaxis

Three females did not approach either stimulus during the trials. The remaining 15 females responded in both trials. For the 15 responsive females, we found FF:FS:SS = 8:4:3. This differed significantly from the null expectation of no preference ($\chi^2_2 = 6.6$, $P = 0.037$). In general, females were twice as likely to approach the Fast stimulus as the Slow stimulus (F:S=20:10). Females also had shorter latencies to approach Fast speakers and spent more time near Fast stimuli than Slow stimuli (Latency_{Fast}: 135.8 ± 20.48 s and Latency_{Slow}: 189.4 ± 20.17 s, Wilcoxon signed-rank test: $t_5 = -10.5$, $P = 0.03$; Time_{Fast}: 105.9 ± 18.09 s and Time_{Slow}: 29.9 ± 6.75 s; Paired t test: $t_5 = 6.71$, $P = 0.001$; Figure 3-3B). Order of stimulus presentation did not influence approach latency or time spent near speakers (Wilcoxon signed-rank tests: $t_5=0.86$, $P = 0.43$ and $t_5=-1.4$, $P = 0.22$, respectively). Time spent near Fast stimuli was positively correlated with variation in performance scores among trials ($R^2 = 0.69$, $F_{1,4} = 9.12$, $P = 0.04$; Figure 3-3C), whereas latency to approach Fast speakers showed a negative association ($R^2 = 0.57$, $F_{1,4} = 5.24$, $P = 0.08$).

Discussion

We documented a vocal performance trade-off in the trills of male Neotropical singing mice and found that androgens play an important role in modulating performance scores. In turn, females showed shorter latencies and spent more time near speakers broadcasting high performance trills that resembled vocalizations of androgen-treated males. These data demonstrate that androgens influence both the structure and efficacy of mouse vocalizations.

Singing mice exhibited a negative relationship between trill rate and frequency bandwidth, suggesting a trade-off similar to that documented in a variety of birds (Podos 1997; Janicke et al. 2008; Podos et al. 2009). Frequency bandwidths are much higher in mice (17-30 kHz) than in birds (1-8 kHz; Podos 1997; Ballentine et al. 2004; Illes et al. 2006; Janicke et al. 2008), reflecting divergent mechanisms of vocal production (Fitch and Hauser 2003). Notably, high frequency sound production did not seem to correspond to large gape widths (Figure 3-2A), suggesting gape-width functions differently across taxa. Indeed, vocal performance trade-offs may not arise from gape width *per se*, but from additional motor constraints governing sound-producing organs (i.e. syrinx and larynx) and surrounding musculature (Suthers and Goller 1997; Nowicki et al. 1992; Podos and Nowicki 2004; Nelson et al. 2005). While the origins of the trade-off in mice remains to be elucidated, such convergence across taxa suggests fundamental constraints on mechanisms of trill production.

Androgens played a strong role in modulating vocal performance in singing mice and appear to be necessary for the proper performance of trills. Both trill rate and frequency bandwidth decreased following castration and administration of empty implants, whereas T and DHT implants maintained both measures. The ability of DHT, a non-aromatizable androgen, to maintain vocalizations suggests that androgens act directly on androgen receptors rather than being aromatized to estrogens. However, administration of aromatase inhibitors would help to clarify the possible contributions of extra-gonadal (e.g. adrenal) sources of aromatizable androgens. To our knowledge, this is the first study to experimentally manipulate androgens levels to assess their impact on vocal performance. Circulating levels of testosterone are correlated with the

number of notes in avian 'rattles,' a phrase type similar in structure to trills and used in aggressive contexts (Galeotti et al. 1997). In singing mice, androgens also modulate aggressive behaviour, spontaneous song rate, and dominant frequency of trills (Pasch et al. 2011). These extensive effects on a suite of behavioural phenotypes suggest that androgens act on motivational centers in the brain as well as the larynx and its surrounding musculature. In anurans, androgens alter the expression of laryngeal myosin heavy chain isoforms that influence the velocity and force of muscular contractions (Fischer et al. 1993). In addition, musculature of both the diaphragm and jaw are known to be androgen-responsive in mammals (Lyons et al. 1986; Prezant et al. 1997; Eason et al. 2000), providing additional possible routes for androgen influences over vocal performance. Future studies that disentangle central and peripheral targets of androgens will be valuable in understanding their dynamic effects on vocal signals that reflect performance.

While androgens had a strong influence on vocal performance in experimental animals in the laboratory, field-captured males showed a significant but weak relationship between body condition and performance score. Such weak correlations between condition and performance are often found in birds (Ballentine et al. 2004; Beebee 2004; Janicke et al. 2008). We speculate that this weak correlation emerges because males modulate vocal effort based on their standing relative to competitors, rather than based on their absolute body condition. In contrast to residual mass, androgen levels have a strong influence over vocal performance because they more accurately reflect a male's resource holding potential (RHP, Parker 1974). Our reasoning draws heavily on the well-established ability of sexual and aggressive

success to elicit testosterone surges in many taxa (Wingfield 1987; Wingfield et al. 1990; Oliveira 2004). Such successes provide a male with direct measures of his ability to monopolize access to resources like mates, space, or food. Androgen responses to success may then act on androgen-responsive tissues in the brain and body to translate individual differences in RHP into an appropriate level of reproductive investment. To assess whether androgens are indeed signaling individual differences in RHP to the body's tissues, it will be useful to correlate circulating levels of androgens with vocal performance measures in the field. In practice, however, the episodic release of androgens may make it difficult to detect relationships between plasma titers and behavior, and measures such as fecal metabolites may prove more stable predictors. It will also be necessary to manipulate both social experience and testosterone surges (e.g. Oyegbile and Marler 2005) to observe the impact each has over vocal performance.

The fact that females showed preference for trills that had higher performance scores provides preliminary evidence that vocal performance may be used in mate choice, though we cannot distinguish among preferences for duration, trill rate, or vocal performance *per se*. We speculate that trill duration may not be as informative as other measures because female birds and frogs tend to prefer longer vocalizations (not shorter ones, as found herein; e.g. Clayton and Prove 1989; Ryan and Keddy-Hector 1992; Neubauer 1999; Gerhardt et al. 2000). Decoupling the importance of vocal performance from trill rate will require additional playback experiments that manipulate frequency bandwidth while holding trill rate constant. Nevertheless, our experiment mirrors avian studies that demonstrate female preference for high performance songs

(Draganoiu et al. 2002; Ballentine et al. 2009; Caro et al. 2010) and recent work in lab mice (*Mus*) showing female approach to male vocalizations used in sexual contexts (Hammerschmidt et al. 2009). More generally, our data add to a growing body of literature suggesting that female mate choice is based upon assessment of male motor performance (reviewed in Byers et al. 2010). The correlation between female responses and performance scores among trials suggests that females not only attend to variation between two given trills, but increase their response to high performance trills independent of the stimulus against which it is compared (Vallet et al. 1998; Ballentine et al. 2004). Understanding how such preferences contribute to reproductive success under more natural conditions will provide important insight into the evolution of male trills.

Our study highlights links between hormones coordinating reproductive behaviours and trade-offs inherent to the production of physically challenging displays. Collectively, our findings support the classification of vocal performance as an index signal, with performance scores putatively reflecting the combination of social and physiological factors that contribute to androgen release (Vehrencamp 2000; DuBois et al. 2008). In turn, females attend to variation in male vocal performance and use it to guide their behaviours. These findings elucidate how male reproductive status can be translated into biologically meaningful variation in signal form.

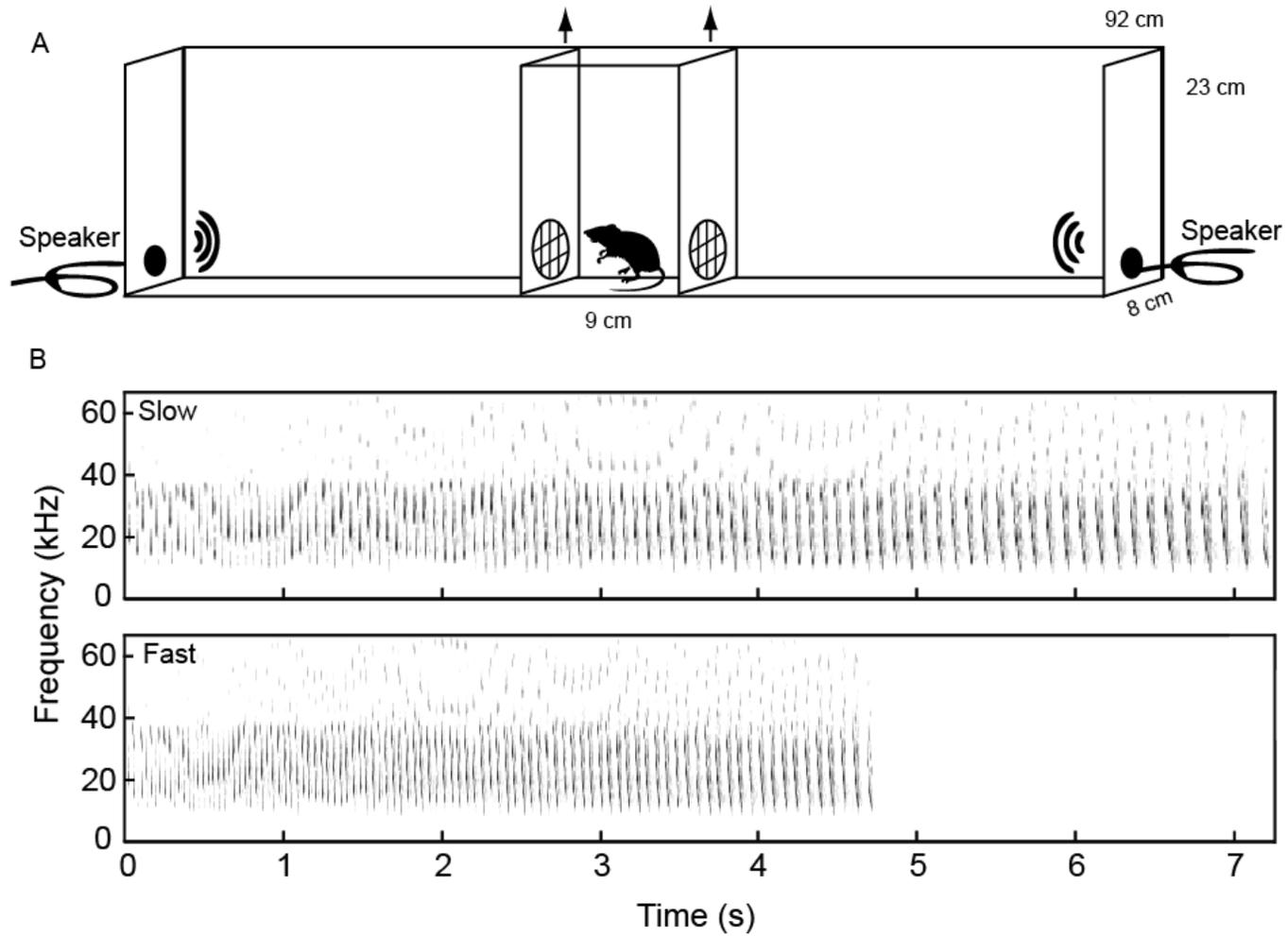


Figure 3-1. Design of female preference experiment. A) Experimental arena and B) spectrograms of Slow and Fast stimuli used in the female preference study. Mouse was redrawn from Reid (1998).

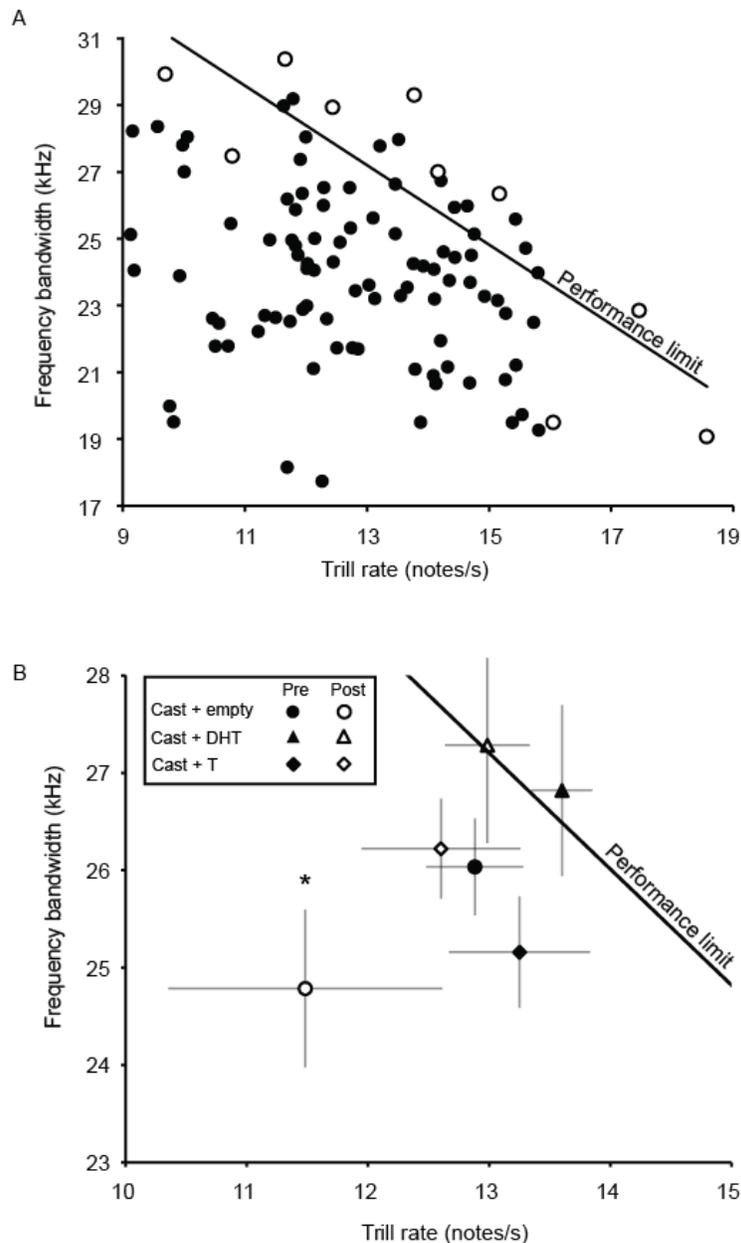


Figure 3-2. Vocal performance in male singing mice. A) Average trill rate (notes/s) vs. frequency bandwidth (kHz) of male *S. teguina* from Costa Rican and Panamanian field populations (filled circles; $N = 102$). The upper-bound regression on trills with the largest frequency bandwidth per 1 note/s bin (open circles) represents a performance limit on trill production. B) Trill rate (notes/s) vs. frequency bandwidth (kHz) of male *S. teguina* pre- and post-treatment ($N = 9$ per group) plotted against performance limit of males from Costa Rican and Panamanian field populations from (a). Error bars are ± 1 SD. * $P < 0.05$ for deviation of performance score (orthogonal distance from upper-bound regression).

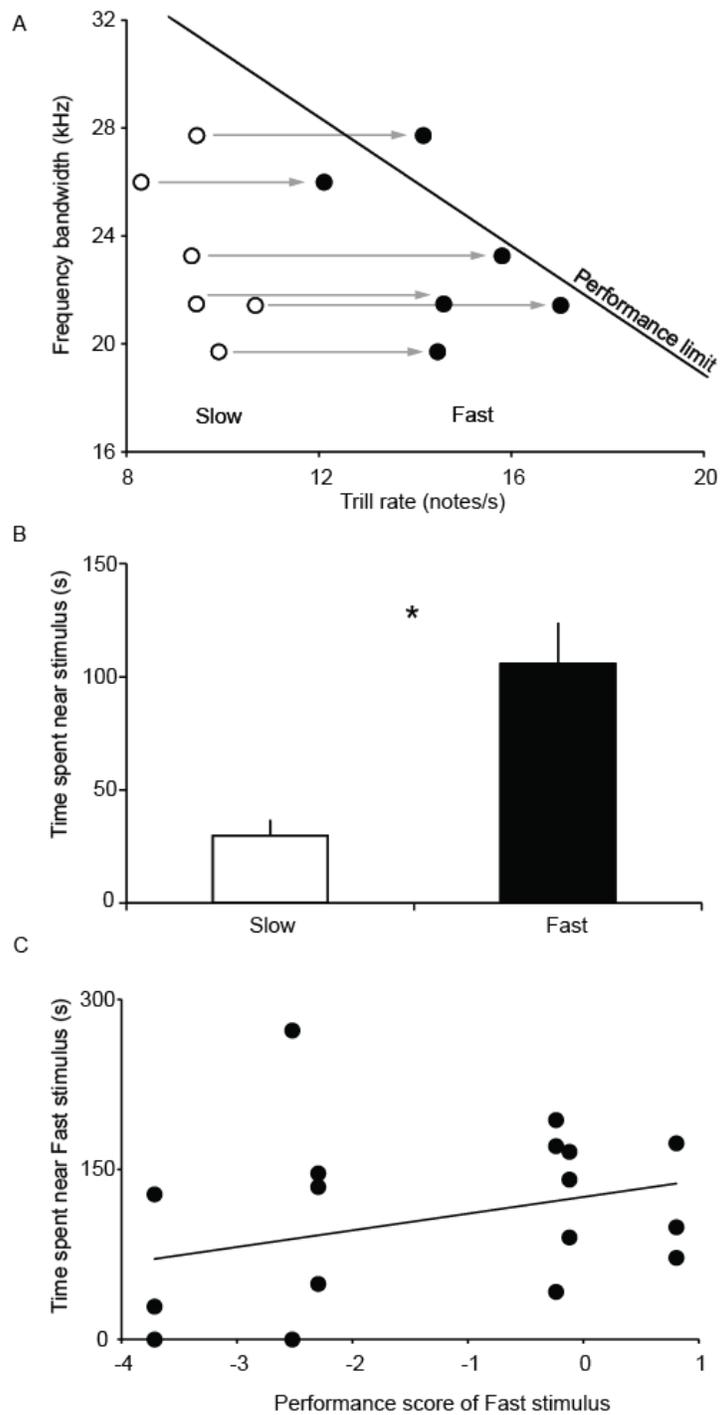


Figure 3-3. Female response to experimentally manipulated male trills. A) Trill rate (notes/s) vs. frequency bandwidth (kHz) of male *S. teguina* from Cerros La Carpintera males ($N = 6$). Arrows illustrate the experimental manipulation of vocal performance from Slow (open circles) to Fast (filled circles) stimuli. B) Time spent near Slow and Fast stimuli ± 1 SE. * $P < 0.05$. C) Amount of time females spent near Fast stimuli against male performance score among trials.

Object 3-1. Audio and video of a male Alston's singing mouse (*S. teguina*) song. Filmed at 100 frames per second and played back at 30 frames per second. (.avi file 11.7 MB)

CHAPTER 4
INTERSPECIFIC DOMINANCE VIA VOCAL INTERACTIONS MEDIATES
ALTITUDINAL ZONATION IN NEOTROPICAL SINGING MICE

Background

Competition for limited resources often results in the evolution of behavioral displays that minimize direct physical combat. In many species, advertisement signals are used to communicate sender identity to a network of potential competitors over long distances (Bradbury and Vehrencamp 1998). By encoding information about resource-holding potential (Parker 1974), signals reduce escalation of aggressive contests and help shape the spatial distribution of animals across landscapes. Thus, ecological spacing patterns are intrinsically linked to communication systems in a variety of taxa.

Signals used to settle aggressive conflicts within species may be an important component of aggression between species (Bradbury and Vehrencamp 1998; Ord and Stamps 2009; Peiman and Robinson 2010). In particular, advertisement vocalizations that mediate competitive interactions among conspecific males can also contribute to interspecific dominance and territoriality when ecologically similar species come into contact (Orians and Willson 1964; Murray 1971, 1981; Rice 1978; Catchpole and Leisler 1986; Prescott 1987; Gerhardt and Huber 2002). In birds, response to heterospecific signals reflects underlying dominance interactions; subordinate species are silenced and/or repelled, whereas dominant species respond aggressively (Robinson and Terborgh 1995; Martin et al. 1996). At small spatial scales, this type of asymmetric vocal dominance can result in local habitat segregation (Martin and Martin 2001a, b). At broader scales, interspecific communication may facilitate competitive exclusion of subordinate taxa across environmental gradients (Robinson and Terborgh 1995; Jankowski et al 2010). Acoustic signaling thus represents an important yet understudied

mechanism mediating interspecific aggression that may contribute to spatial segregation at species limits.

The abrupt replacement of species along altitudinal gradients has long fascinated biologists (Humboldt 1807; Merriam 1895; Shreve 1915, 1922) and provided an opportunity to study how biotic factors limit geographic distributions (Brown 1971; Heller 1971; Heller and Gates 1971; Chappell 1978; Hairston 1980a, b, 1981; Repasky and Schluter 1994; Carothers et al. 2001; Twomey et al. 2008). Interspecific competition is posited to be a major factor delimiting the range edge of closely related parapatric species (Bull 1991), though most evidence is based on expansion of altitudinal ranges in the absence of putative competitors (Lack and Southern 1949; Diamond 1970, 1973; Terborgh 1971; Terborgh and Weske 1975; Mayr and Diamond 1976; Remsen and Graves 1995; but see Cadena 2007 and Cadena and Loiselle 2007). However, recent experimental work in Neotropical birds suggests that interspecific aggression maintains altitudinal range margins (Jankowski et al. 2010), highlighting the importance of behavioral interactions in limiting species distributions.

Neotropical singing mice (*Scotinomys*) are diurnal, insectivorous rodents distributed throughout the mountains of Central America. Alston's singing mouse (*S. teguina*; 9-12 g) is a widespread species inhabiting mid- to high-altitude cloud forest, forest edge, and abandoned pastures from southeastern México to western Panamá (1000-2900 m), whereas the Chiriquí singing mouse (*S. xerampelinus*; 13-15 g) is restricted to the highest forested summits and páramo of Costa Rica and Panamá (2200-3300m; Wilson and Reeder 2005; Hooper and Carleton 1976; Figure 4-1). The two ecologically similar congeners occupy parapatric distributions in the Cordillera de

Talamanca and Cordillera Central, where the upper distribution of *S. teguina* contacts the lower distribution of *S. xerampelinus* between 2200-2900 m (Enders and Pearson 1939; Hooper 1972; Hooper and Carleton 1976). Interspecific competition and species differences in thermal tolerance are hypothesized to maintain this narrow contact zone (Hill and Hooper 1971; Hooper and Carleton 1976). Males of both species commonly sing species-specific trills (Miller and Engstrom 2007; Campbell et al. 2010) that are modulated by androgens and used in mate attraction and male-male aggression (Pasch et al. 2011a, b; Object 4-1). Vocalizations of *S. teguina* are long (4-7 s), decelerating trills of moderate power (54 dB SPL at 1 m) and high dominant frequencies (22-26 kHz), whereas *S. xerampelinus* trills are shorter (1-2 s), louder (63 dB SPL at 1 m), and lower in frequency (16-20 kHz: Campbell et al. 2010; Object 2-1 and Object 4-2).

The first goal of this study was to identify the altitude of species replacement along three slopes in the Cordillera de Talamanca and Cordillera Central. We placed data loggers at replacement zones to provide insight into the role of temperature in limiting species distributions. Similar temperature regimes at replacement zones on different mountains would suggest that abiotic factors are important in limiting one or both species. Our second goal was to evaluate the importance of species interactions in mediating altitudinal range limits. If interspecific interactions mediate species replacement, we predicted that a) species would exhibit interspecific aggression in staged behavioral trials and b) removal of one species would result in competitive release of the opposite species (Connell 1983). Finally, if interspecific vocal communication contributes to species replacements, we predicted that response to playback of heterospecific song would reflect underlying dominance interactions.

Materials and Methods

Altitudinal Replacement Zones and Temperature Regimes

In June-August 2007, we established trapping transects to demarcate zones of altitudinal replacement on two mountain slopes in Costa Rica (Cerro de la Muerte-CM; Volcán Irazú-VI) and one slope in Panamá (Volcán Barú [VB]; Figure 4-1). Hooper and Carlton's (1976) original account of contact zones helped guide the selection of study sites. A 200 m x 30 m grid of 44 traps (spaced 20 m perpendicular and 10 m parallel to the cline, respectively) was placed in the understory for 20 days at each site. If *S. teguina* was captured, the entire grid was moved 50 m upslope the following day. In addition, sporadic trap lines were placed at altitudes \pm 300 m from the trapping grid to validate species distributions. Sherman live-traps (Model SFA) were baited with peanut butter and oats and checked daily. We defined the altitude of replacement as the trapping grid where *S. xerampelinus* was captured and *S. teguina* was not captured over the course of 5 trap days.

To provide a general estimate of ambient temperatures experienced by each species, temperature loggers (Hobo Pendant, Model UA-001-64, Onset Computer) were placed in the understory at replacement zones to collect ambient temperature every hour for 1 to 3 years. At one site (VI), we placed a temperature logger at the top of the mountain to characterize temperature regimes where *S. xerampelinus* occurs in allopatry. Along the same slope, we estimated the lowest altitude of *S. teguina* occurrence by broadcasting conspecific song at 100 m altitudinal intervals. Response to song indicated species presence (Hooper and Carleton 1976). Broadcast songs were randomly selected from laboratory recordings of *S. teguina* captured higher on the same mountain (Campbell et al. 2010). We played stimuli at 54 dB SPL at 1 m from a Pioneer

TS-250 speaker (flat frequency response 6–40 kHz) via an external soundcard (Edirol FA-66) connected to a laptop running Adobe Audition. Songs were broadcast at 3 min intervals followed by 2 min of silence for 20 min between 0700-1000. A temperature logger was placed at the lowest site where the vocal response of *S. teguina* was recorded.

Interspecific Behavioral Trials

To examine whether singing mice exhibited interspecific aggression, we staged interspecific interactions between adult males of *S. teguina* and *S. xerampelinus* captured at the contact zone at CM and VI in June-August 2007 ($n = 9$ trials/site). One individual of each species was placed on opposite sides of an 80 x 50 x 40 cm arena separated by an opaque partition for 48 hrs after capture. The arena was isolated in an open-air laboratory on site and animals were fed *ad libitum* with cat chow and water. The partition was removed between 0700-0900 to allow animals to interact for 10 min. We videotaped all trials and scored the number of aggressive attacks and latency to attack for each species.

Reciprocal Removal Experiment

If interspecific interactions mediate species replacement, we predicted that removal of one species would result in competitive release of the opposite species in the form of immigration. In May-August 2008, we performed a reciprocal removal experiment at the zone of replacement on VI, which coincided with a transition from secondary forest scrub (solely occupied by *S. teguina*) to primary forest (solely occupied by *S. xerampelinus*; Appendix A). We established twelve 32 m x 24 m plots of 20 traps spaced 8 m apart in a rectangular grid. We paired control and removal plots for a given resident species (*S. teguina* or *S. xerampelinus*) with 3 replicates per species. Pairs of

control-removal plots were spaced 100 m apart and were separated from all other paired plots by > 500 m. The vertical distance between *S. teguina* and *S. xerampelinus* plots was ca. 30 m and spanned 15 m in elevation. Trapping was conducted for 3 consecutive days and every 5 days thereafter over 9 weeks. On control plots, resident animals were captured, marked for unique identification, and released on site. On removal plots, individuals of the resident species were captured, uniquely marked, and either transplanted 2 km or used in laboratory playback experiments (below); individuals of the colonizing immigrant species were uniquely marked and released on site. Temperature loggers were placed in the center of all plots to record temperature every hour for 14 days. Temperatures were averaged across control and removal plots for all replicates.

Field Playback Experiments

If vocal communication contributes to species replacement, we predicted that response to playback of heterospecific song would reflect underlying dominance interactions. During the removal experiment, we identified traps on control plots where resident males were captured most often. At the end of the experiment, we visited those trap sites ($n = 8$ per species) between 0600-1000 and waited 10 min before initiating playbacks. Three types of stimuli were played during each trial: Control stimulus, heterospecific song, and conspecific song. Songs used in playback experiments were recorded from adult males of the same population during the previous year (Campbell et al 2010). We selected at random 2 songs from each of 8 males per species and used songs of a different male for each trial. Control stimuli were generated in Adobe Audition using band-pass filtered white noise that matched the spectral envelopes, duration, and root mean square (RMS) amplitude of corresponding conspecific songs. A Bruel and

Kjær sound level meter (Type 2219) and calibrator (Type 4230) were used to calibrate the amplitude of stimuli to match average songs of *S. teguina* (54 dB at 1 m) or *S. xerampelinus* (63 dB at 1 m).

Each playback trial lasted 26 min and was recorded with a Sony PCM-50 digital recorder. We began with a 5 min pre-stimulus period to record background levels of singing. At 5 and 6 min we broadcast each of two heterospecific songs, and recorded the number of songs evoked over a five-minute interval (7-12 min); at 12 and 13 min we broadcast each of two control stimuli and recorded responses for five minutes (14-19 min); and at 19 and 20 min we broadcast each of two conspecific songs and recorded responses from 21-26 min. In half of the trials, control stimuli were presented at 5 and 6 min, and heterospecific stimuli at 12 and 13 min. Conspecific songs were always played last to avoid carryover effects that were apparent in preliminary studies (also see Martin and Martin 2001b). Species identity of responding individuals was verified from analysis of digital recordings, with *S. teguina* songs being consistently longer than *S. xerampelinus* songs at VI (5.37 ± 0.95 s vs. 1.77 ± 0.41 s, respectively; Campbell et al. 2010).

Laboratory Playback Experiments

To validate playback experiments in the field and quantify animal movement associated with vocal behavior, we used adult males captured on removal plots to perform playbacks in a controlled laboratory setting. Animals were housed singly in 28 x 28 x 28 cm PVC-coated wiremesh cages and isolated in anechoic chambers for 24 hrs prior to initiating trials between 0600-1000. Individuals of each species were assigned randomly to playback of 1 of 3 stimuli: Control stimuli, heterospecific song, or conspecific song (*S. teguina*: $n = 8$ per treatment; *S. xerampelinus*; $n = 5$ per treatment).

We selected 3 songs from each of 4 individuals of each species from laboratory recordings of the same population (Campbell et al. 2010). Control stimuli were synthesized by generating white noise bounded within species-specific frequency envelopes (*S. teguina* 10-42 kHz, *S. xerampelinus* 10-38 kHz). We played stimuli at 50 dB SPL at 1 m from one of four randomly selected Pioneer TS-250 speakers placed on each side of the mesh cage. Mice were recorded for 20 min to obtain pre-stimulus song rate. At 20, 21, and 22 min, we broadcast each of 1 song followed by 2 minutes of silence. The sequence was repeated three times with songs from different males. For control treatments, the single control stimulus was broadcast at the same rate as conspecific and heterospecific song treatments. We recorded the total number of songs emitted by focal animals in the 20 min period following the initial stimulus. We also calculated the amount of time animals spent on the half of the cage that held the speaker emitting the stimulus.

Statistical Analyses

We analyzed response to the removal experiment using a generalized linear model (GLM) with a Poisson distribution (log link) performed in SAS/STAT v 9.2 (SAS Institute 2008). Explanatory variables included species identity of resident animals (*S. teguina* or *S. xerampelinus*), species identity of colonizing animals (*S. teguina* or *S. xerampelinus*), and an interaction term. Plot replicate was initially incorporated as a random effect but was removed because the model did not converge, indicating little variance among plots (Bolker et al. 2008; SAS Institute 2008). For each species, the response variable was calculated by subtracting the number of colonizers on control plots from their paired removal plots following the initial 3-day trapping period.

To analyze response to reciprocal playback experiments in the field, we used a Poisson distributed GLM. Individual was initially coded as a random effect but was removed because the estimated variance was zero (Bolker et al. 2008; SAS Institute 2008). The number of songs did not differ between prestimulus and control periods for either species (Kruskal-Wallis nonparametric ANOVA, $\chi^2 = 0.53$ and 0.27 , $df = 1$, $P = 0.46$ and 0.60 for *S. teguina* and *S. xerampelinus*, respectively). Therefore, we removed the prestimulus period from our model. Order effects were not significant ($P = 0.49$) and were excluded from the model, resulting in stimulus type (control, conspecific, or heterospecific), species identity (*S. teguina* or *S. xerampelinus*), and an interaction term as explanatory variables. Contrast statements were used to test for significant differences among groups after adjusting for overdispersion using the Pearson χ^2 statistic (Littell et al. 2002).

To analyze responses to playbacks in the laboratory, a multi-factor ANOVA was applied to ranked data using the Scheirer–Ray–Hare technique (Sokal and Rohlf 1995, Dytham 1999). The number of songs evoked post-stimulus was subtracted from pre-stimulus levels to obtain the response variable; species identity, stimulus type, and an interaction term were included as explanatory variables. Kruskal-Wallis tests were then used to test for significant differences among treatment groups. To test differences among treatment groups in time spent near speakers, we used ANOVA followed by Tukey's HSD-corrected post-hoc comparisons.

Results

Altitudinal Replacement Zones

The altitude of replacement differed among mountains (VI: 2920 m; CM: 2610 m; VB: 2150 m; Figure 4-2A), yet temperature regimes that characterized replacement

zones were qualitatively similar (Figure 4-2B). Daily temperature profiles showed little variation and decreased with increasing altitude. Across their altitudinal distributions, *S. xerampelinus* experiences a narrower range and cooler temperatures than does *S. teguina* (*S. xerampelinus*: 7.3 – 9.9 °C; *S. teguina*: 9.9 – 18.7 °C; Figure 4-2B). On the mountain with the highest altitude of replacement (VI), the replacement zone was concomitant with a change in vegetation from early succession scrubland at lower elevations to mature forest at higher elevations. Such vegetation transitions were not readily apparent at other localities.

Interspecific Behavioral Trials

In 17 of 18 trials, *S. xerampelinus* initiated more attacks than *S. teguina* (4.8 ± 0.9 and 0.67 ± 0.3 attacks; $t = 4.91$, $df = 17$, $P < 0.0001$; Figure 4-3A). In one trial, neither *S. teguina* nor *S. xerampelinus* exhibited aggression. Similarly, *S. xerampelinus* showed shorter latencies to attack than did *S. teguina* (156.9 ± 41.6 and 442.4 ± 55.6 s, respectively; Wilcoxon signed-rank = -59, $P = 0.001$; Figure 4-3B).

Reciprocal Removal Experiment

We trapped for 26,880 trap-hours over 9 weeks and captured 121 *S. teguina* (removed 65) and 71 *S. xerampelinus* (removed 34). *S. teguina* plots were warmer and showed more diel variation than *S. xerampelinus* plots (*S. teguina*: 11.5 ± 3.65 °C; *S. xerampelinus*: 9.4 ± 0.78 °C; Wilcoxon signed-rank test, -14771, $P < 0.0001$; Figure 4-4A). We found an asymmetrical response to removals, indicated by a significant interaction between species identity of resident and colonizing animals ($\chi^2 = 3.83$, $df = 1$, $P = 0.05$; Figure 4-4B). *S. teguina* colonized *S. xerampelinus* removal plots within 3

weeks and remained there for the duration of the study ($n = 19$), whereas no *S. xerampelinus* colonized *S. teguina* removal plots (Figure 4-4C, D).

Field Playback Experiments

We found a significant interaction between stimulus type and species identity ($\chi^2 = 32.53$, $df = 2$, $P < 0.001$). Compared to control stimuli, *S. teguina* were more likely to sing in response to conspecifics and less likely to respond to heterospecifics ($\chi^2 = 6.28$ and 25.65 respectively, $df = 1$, $P < 0.01$; Figure 4-5A). Conversely, *S. xerampelinus* were more likely to sing in response to both conspecifics and heterospecifics compared to control stimuli ($\chi^2 = 5.07$ and 3.14 , $P = 0.02$ and 0.07 respectively; Figure 4-5B).

Laboratory Playback Experiments

Similar to field playback experiments, we found a significant interaction between stimulus type and species identity ($\chi^2 = 9.05$, $df = 2$, $P < 0.02$). *S. teguina* were more likely to sing in response to conspecifics and less likely to respond to heterospecifics when compared to control stimuli ($H = 10.52$ and 8.68 respectively, $df = 1$, $P < 0.003$; Figure 4-6A). Accordingly, *S. teguina* spent more time near the speaker broadcasting conspecific stimuli ($73 \pm 6\%$) and less time near heterospecific stimuli ($12 \pm 6\%$) compared to controls ($44 \pm 11\%$, $F_{2,21} = 14.45$, $P > 0.001$; Figure 4-6B). *S. xerampelinus* showed a tendency to respond to both conspecifics and heterospecifics, but our sample size was insufficient to detect differences (both $H = 2.22$, $df = 1$, $P = 0.13$; Figure 4-6A). However, *S. xerampelinus* spent more time near speakers broadcasting conspecific and heterospecific stimuli ($70 \pm 9\%$ and $73 \pm 6\%$, respectively) compared to controls ($32 \pm 4\%$, $F_{2,12} = 11.67$, $P = 0.001$; Figure 4-6B).

Discussion

We investigated patterns of altitudinal zonation in Neotropical singing mice and found similar temperature regimes across different replacement sites, indicating that abiotic factors may be important in limiting one or both species. Subsequent studies revealed that the larger, higher altitude species, *S. xerampelinus*, was behaviorally dominant to the smaller, lower altitude *S. teguina*. Removal of *S. xerampelinus* resulted in colonization of *S. teguina* into cooler microclimates; conversely, *S. xerampelinus* did not colonize *S. teguina* removal plots characterized by warmer, more variable temperatures. Similarly, asymmetrical response to playback experiments in the field and laboratory indicated that acoustic communication reflects underlying dominance interactions; *S. xerampelinus* responded to heterospecific song, whereas *S. teguina* was silenced by song of dominant heterospecifics. Our findings are consistent with aggressive exclusion of *S. teguina* from cooler environments at higher elevations. The study highlights how behavioral interactions mediated by interspecific communication can influence geographic distributions of ecologically similar congeners.

The altitude at which *S. xerampelinus* replaces *S. teguina* differed greatly across study sites. However, similar temperature regimes at each site suggested an important role for abiotic factors in contributing to species range limits. Indeed, the strong climatic stratification along tropical mountain slopes is thought to limit species distributions via selection on physiological tolerance to temperature (Janzen 1967; Ghalambor et al. 2006; Tewksbury et al. 2008; McCain 2009). Hooper and Carleton (1976) found that the contact zone between *S. xerampelinus* and *S. teguina* tracked abiotic conditions as determined by the interaction of topography (e.g. slope, aspect) and climatic patterns.

For example, the replacement zone decreases from 2930 m to 2350 m on the eastern slopes of VI, where moisture-laden wind and cloud cover provide conditions for epiphyte-rich vegetation that confer cool microclimates suitable to *S. xerampelinus* (Hooper and Carleton 1976). We confirmed this pattern quantitatively, and proceeded to test whether biotic interactions contribute to the abrupt replacement zones.

At the highest zone of contact (VI), the replacement of *S. teguina* by *S. xerampelinus* coincided with an abrupt transition between two vegetation types. Here, temperature regimes differed substantially across a small spatial scale (Figure 4-4). This pattern suggests that species differences in distribution may be driven by habitat rather than altitude *per se*. However, such vegetation transitions occur commonly at lower elevations where *S. teguina* inhabits a wide variety of vegetation types, including primary and secondary forest, and abandoned pastures. Furthermore, *S. xerampelinus* is not restricted to primary forest but occurs in páramo and forest edge on the highest peaks of the Cordillera de Talamanca (Hooper 1972; Hooper and Carleton 1976). In our study, *S. teguina* rapidly colonized cooler *S. xerampelinus* plots, whereas *S. xerampelinus* showed no colonization into warmer *S. teguina* plots. These data provide evidence that *S. xerampelinus* responds to abiotic conditions and/or other factors that correlate strongly with temperature regimes. Our findings mirror results of a laboratory study investigating species differences in temperature tolerance (Hill and Hooper 1971). While both species showed thermal tolerances consistent with temperature regimes commonly experienced at native altitudes, experimentally elevating ambient temperatures to 35 °C resulted in death of 66% (2 of 3) of *S. xerampelinus* vs. 0% (0 of 3) of *S. teguina*. In contrast, only 12.5% (1 of 8) of *S. teguina* and 0% (0 of 7) of *S.*

xerampelinus died when ambient temperatures were lowered to 0-5 °C (Hill and Hooper 1971). Despite small sample sizes, the data indicates that *S. teguina* is more able to acclimate to a wider range of ambient temperatures. In concert with our study, we infer that *S. xerampelinus* is relegated to cold summits, whereas *S. teguina* is less sensitive to prevailing abiotic conditions and can inhabit cooler environments in the absence of dominant competitors.

Interference competition is a predominant mechanism mediating interspecific interactions among small mammals, especially insectivores (Grant 1972; Dickman 1991; Rychlik and Zwolak 2006). In many communities, ecologically similar species maximize foraging efficiency by exploiting similar prey items and dominance status is dictated by body size (Morse 1974; Dickman 1991; Martin and Martin 2001b). Smaller subordinate species tolerate interspecific interference because the benefits of gaining temporary access to resource-rich microenvironments outweigh the costs of vigilance and evasion. In contrast, the cost of interference to larger dominant species is low compared to the benefits of exclusive access to prey-rich microenvironments (Chappell 1978; Dickman 1991). Consequently, smaller-bodied species are aggressively repelled by larger-bodied congeners and show rapid competitive release upon their removal (Dickman 1986a, b, 1988). In our system, the larger *S. xerampelinus* feeds primarily on nutrient-rich larval insects, whereas *S. teguina* includes a greater proportion of seed, berries, and fibrous plant materials (Hooper and Carleton 1976). *S. xerampelinus* is also behaviorally dominant, as demonstrated by more aggression and shorter latencies to attack in interspecific trials. Moreover, subordinate *S. teguina* exhibited rapid colonization into *S. xerampelinus* removal plots, whereas *S. xerampelinus* showed no

response to *S. teguina* removal. Such asymmetric interactions suggest that *S. teguina* is limited by interference (“encounter competition”; Schoener 1983) from *S. xerampelinus*, presumably for access to resource-rich microenvironments. Size-dependent interspecific dominance is prevalent in other taxa and may be a common biotic mechanism mediating geographic range limits (Morse 1974; Persson 1985; Glazier and Eckert 2002).

Our findings mirror a widespread phenomenon in biology reflecting asymmetric patterns of interspecific dominance relative to physiological tolerance, especially among closely related species that segregate along environmental gradients (e.g. barnacles: Connell 1961; birds: Martin and Martin 2001a; bees: Bowers 1985; chipmunks: Heller 1971, Chappell 1978; crayfish: Bovbjerg 1970; fish: Hixon 1980; salamanders: Jaeger 1971a, b; Griffis and Jaeger 1998). Dominant species are often unable to live in areas occupied by subordinate species, whereas subordinate species can thrive in a variety of habitats but are excluded from areas occupied by dominant species (Morse 1974). In small mammals, this pattern is associated with species differences in geographic range size and degree of specialization (Glazier and Eckert 2002). Dominant species tend to have restricted geographic ranges and evolve in stable environments that favor ecological specialization, which in turn may select for higher aggression to defend narrow niches from invasion by generalists (Miller 1967; Morse 1974; Colwell and Fuentes 1975; Glazier 1980). Understanding the ubiquity of this pattern will be important in the attempt to model species responses to atmospheric warming. For example, rising temperatures are predicted to force montane species upward to eventual extinction, placing tropical mountaintop organisms at high risk (Colwell et al. 2008; Lenoir et al.

2008). Competitive ability may contribute to resistance against this 'escalator effect' and account for variation in species responses to climate change (Davis et al. 1998; Marris 2007; Moritz et al. 2008; Jankowski et al. 2010).

Role of Acoustic Communication in Interspecific Interactions

Acoustic communication is a common mechanism mediating intrasexual competition in numerous taxa. In insects and anurans, advertisement signals contribute to inter-male spacing as males aggressively compete for females (Gerhardt and Huber 2002). In birds and fish, vocalizations help deter intruder entry into territories containing limited resources in addition to mates (Collins 2004; Bass and Ladich 2008). When ecologically similar species come into contact, heterospecific aggression can result in interspecific territoriality, and response to reciprocal playbacks reflects underlying competitive interactions (Robinson and Terborgh 1995; Martin and Martin 2001b). In this study, we found that *S. xerampelinus* responded to both conspecifics and heterospecifics. Conversely, *S. teguina* responded to conspecifics but were silenced by heterospecific song. This pattern matches asymmetric dominance interactions observed in the laboratory and competitive release by *S. teguina* in response to *S. xerampelinus* removal in the field. Insectivores (e.g. shrews and moles) and other insectivorous small mammals aggressively defend territories containing sparse and slowly renewing food supplies (Hawes 1977; Dickman 1986a, b). In *S. xerampelinus*, females and some males exhibit exclusive use of core areas consistent with territoriality (Blondel et al. 2009). Future work will need to characterize the mating systems of both species to fully understand how social organization contributes to vocal interactions at the range edge. Likewise, replacement of speakers in areas where *S. xerampelinus* is removed will elucidate whether songs enforce species limits by deterring *S. teguina* entry.

Heterospecific playback experiments to *S. teguina* in allopatry and manipulation of dominance status in the laboratory will also provide insight into whether aggressive experience is a necessary component of vocal discrimination, as appears to be the case in birds (Jankowski et al. 2010).

Conclusion

Our study highlights the interaction of physiological and behavioral factors in mediating species range limits. Asymmetric patterns of interspecific dominance relative to physiological tolerance appear to be a common phenomenon when ecologically similar species segregate along environmental gradients. Moreover, our findings suggest that asymmetries in interspecific communication reflect underlying dominance interactions and contribute to spatial segregation at the range edge. Interspecific communication may be an important yet overlooked mechanism structuring animal communities.

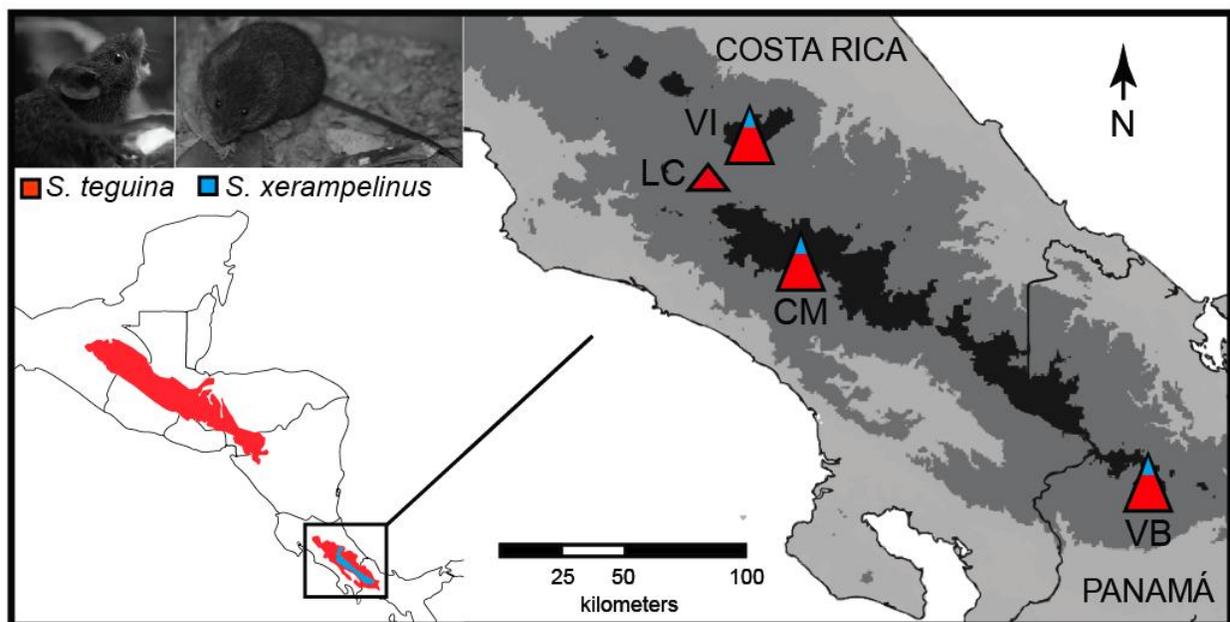


Figure 4-1. Latitudinal and altitudinal distributions of Neotropical singing mice (*Scotinomys*). *S. xerampelinus* (blue) replaces *S. teguina* (red) on the highest mountains of Costa Rica and Panamá. VI- Volcán Irazú, CM- Cerro de la Muerte, VB- Volcán Barú.

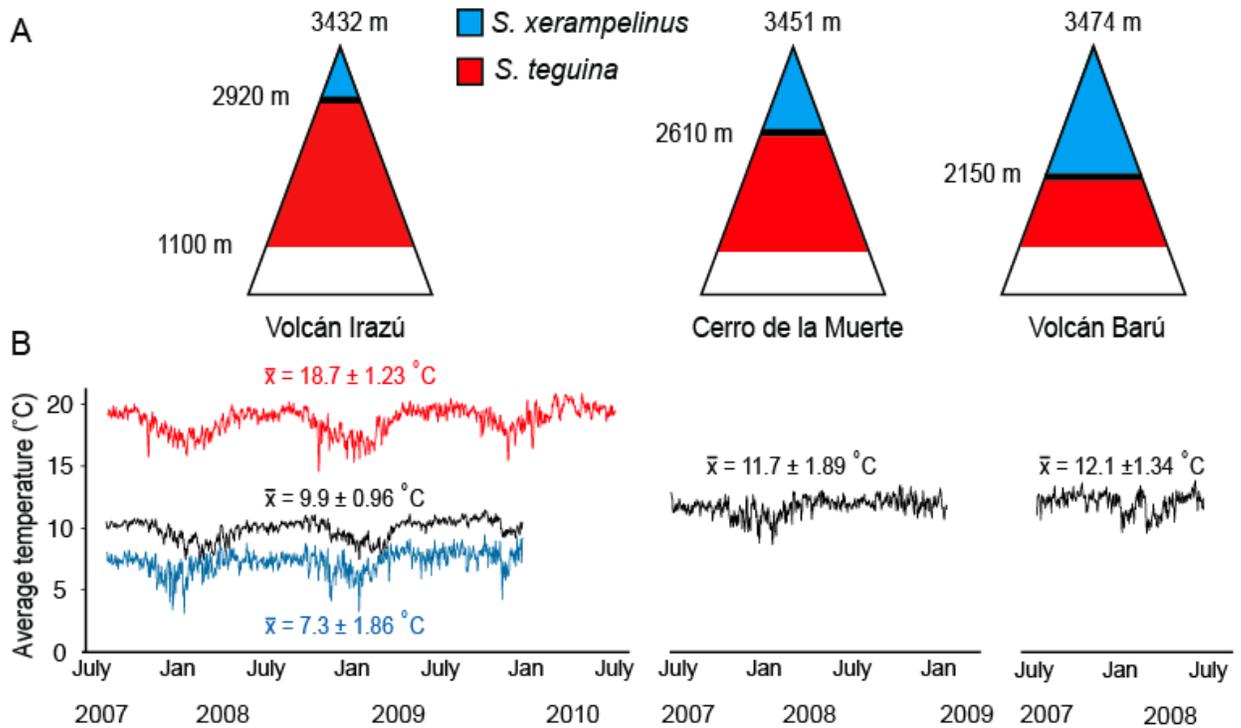


Figure 4-2. Altitudinal distributions and temperature regimes of Neotropical singing mice
 A) Altitude of replacement (black line) on two mountains in Costa Rica (Volcán Irazú and Cerro de la Muerte) and Panamá (Volcán Barú). B) Average temperature regimes at the site of replacement (black). At Volcán Irazú, average temperature regimes of *S. xerampelinus* (blue) and *S. teguina* (red) in allopatry.

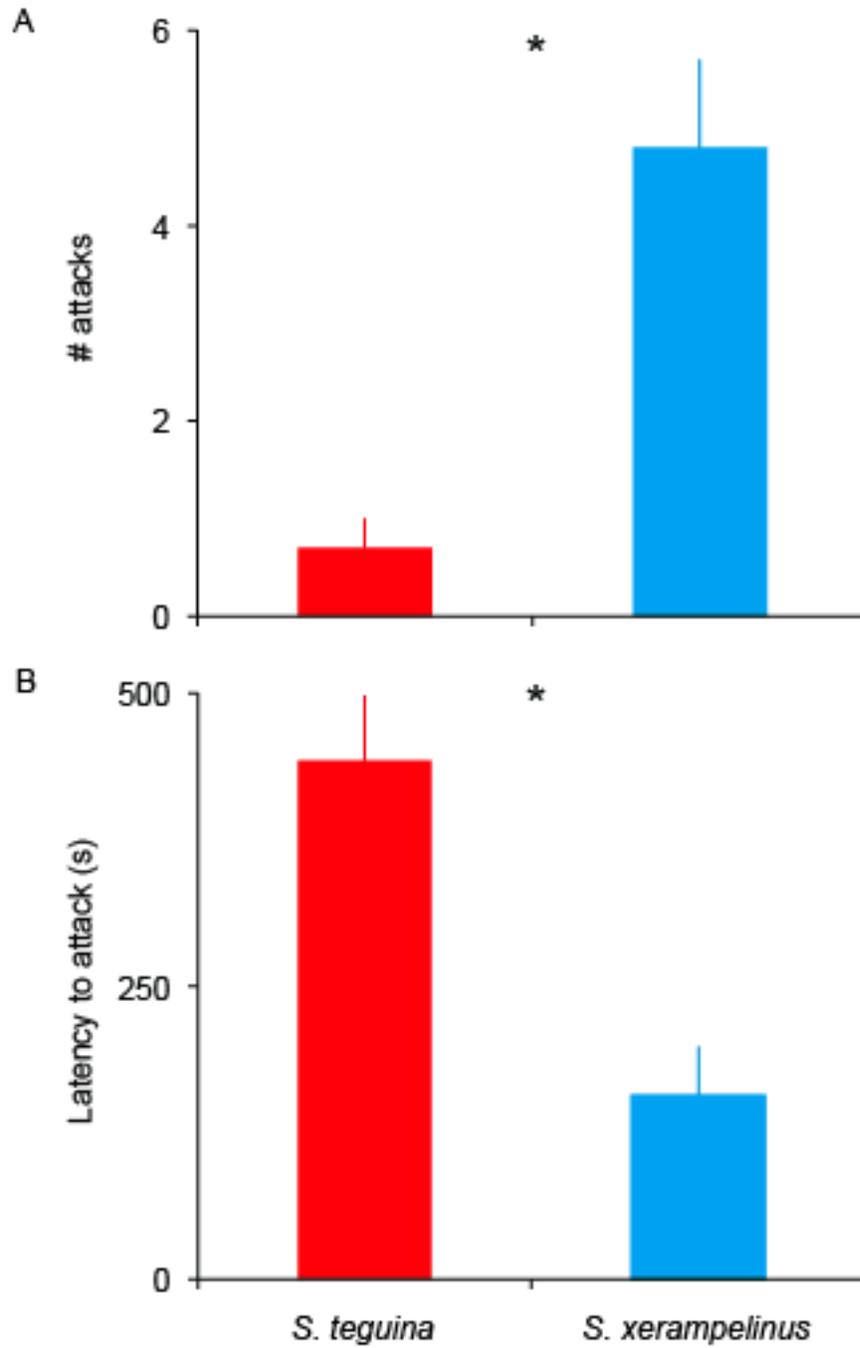


Figure 4-3. Interspecific aggression in Neotropical singing mice. A) The number of attacks initiated by both species during staged trials. B) The latency to attack by both species during staged trials. Error bars are ± 1 s.e. * $P < 0.001$

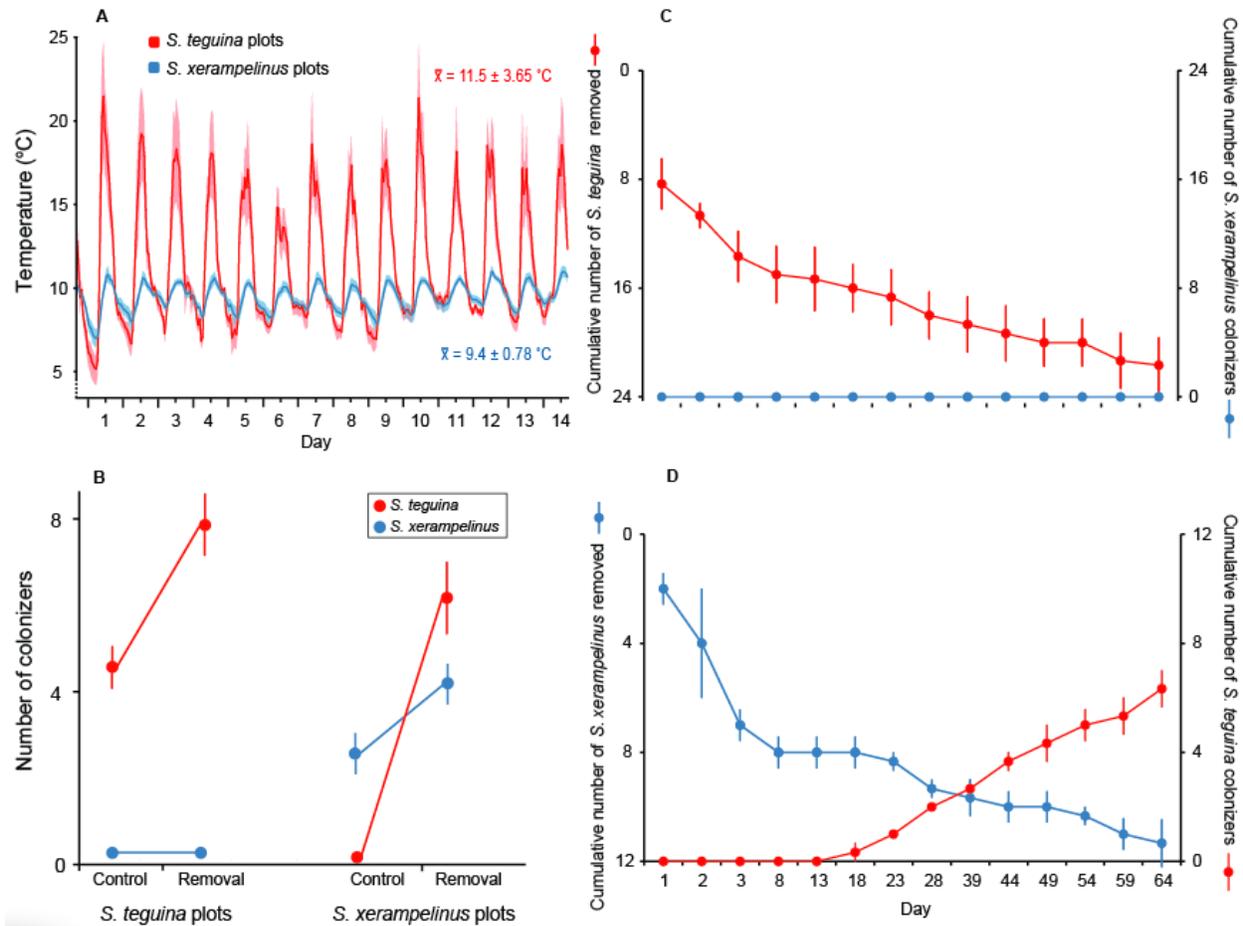


Figure 4-4. Reciprocal removal experiment on Volcán Irazú, Costa Rica. A) Average temperature regimes on *S. xerampelinus* (blue) and *S. teguina* (red) plots. Shading represents ± 1 s.e. B) Average number of colonizers on control and removal plots for *S. teguina* and *S. xerampelinus*. C) Accumulation plot depicting the number of *S. xerampelinus* (blue) colonizing *S. teguina* removal plots as *S. teguina* (red) is removed over time (days). D) Accumulation plot depicting the number of *S. teguina* (red) colonizing *S. xerampelinus* removal plots as *S. xerampelinus* (blue) is removed over time (days). Error bars are ± 1 s.e.

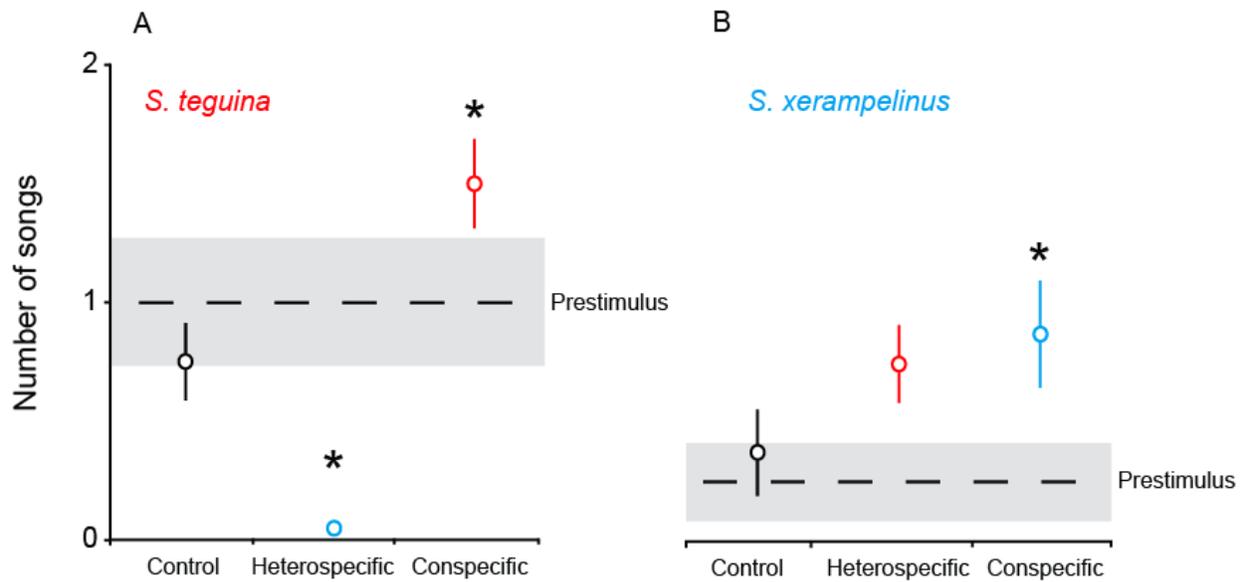


Figure 4-5. Response to playback trials in the field at Volcán Irazú, Costa Rica. A) Number of songs produced by *S. teguina* (red) to playback of control, conspecific, and heterospecific (blue) stimuli. B) Number of songs produced by *S. xerampelinus* (blue) to playback of control, conspecific, and heterospecific (red) stimuli. Dashed line and shading represents the number of songs produced by focal males prior to playback of stimuli. Error bars are \pm 1 s.e. * $P < 0.05$

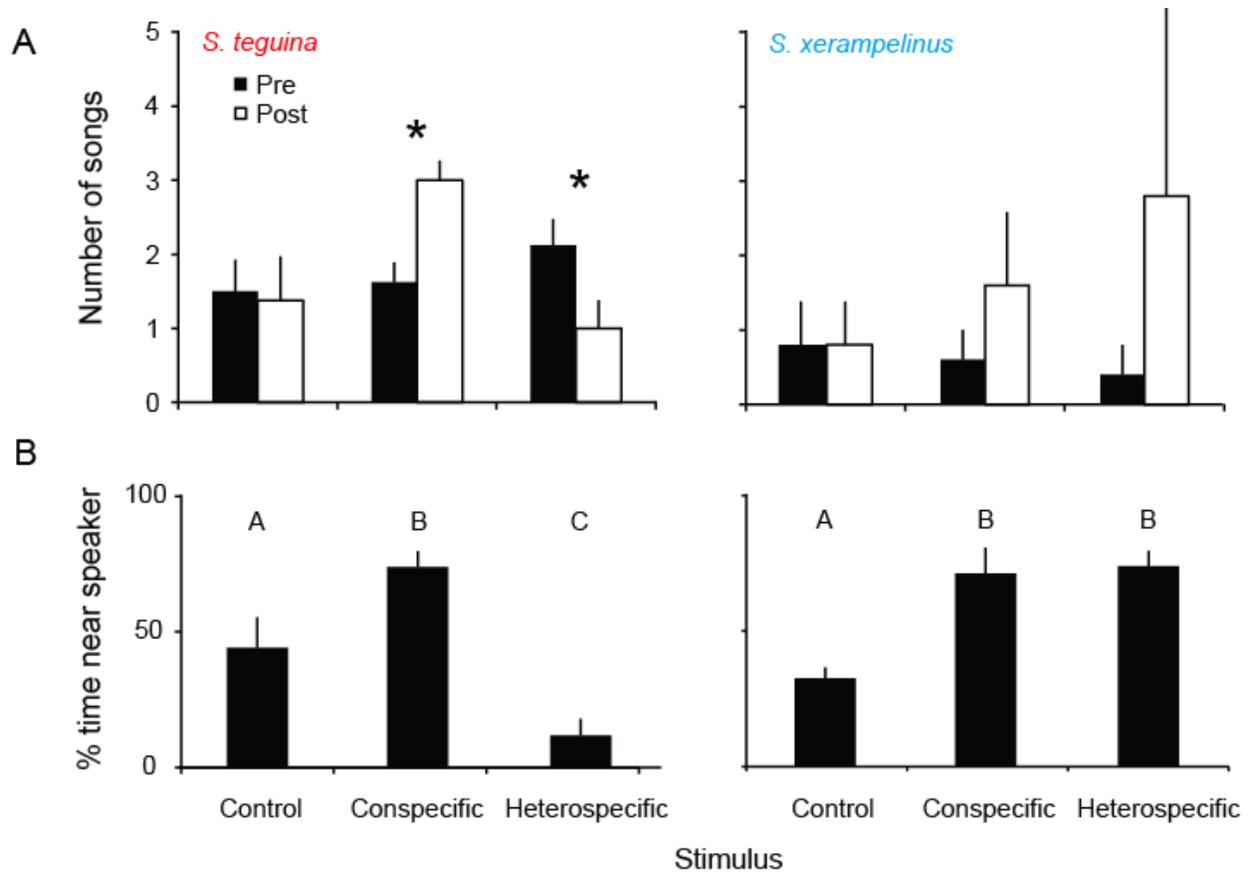


Figure 4-6. Response to playback trials in the laboratory at Volcán Irazú, Costa Rica. A) Number of songs produced by *S. teguina* and *S. xerampelinus* pre- (shaded bars) and post- (open bars) playback of control, conspecific, and heterospecific stimuli. B) Percent time spent near speakers broadcasting sound stimuli. Error bars are ± 1 s.e. * $P < 0.05$. Letters not sharing the same letter are significantly different.

Object 4-1. Vocal response of a male Alston's singing mouse (*S. teguina*) to playback of conspecific song. (.avi file 20 MB)

Object 4-2. Song of a male Chiriquí singing mouse (*S. xerampelinus*) recorded at 96 kHz. (.wav file 0.49 MB)

CHAPTER 5
AGONISTIC CHARACTER DISPLACEMENT IN SOCIAL COGNITION IN A TROPICAL
MOUSE

Background

Interspecific aggression is a prevalent but poorly studied phenomenon that may shape niche use in a variety of taxa (Ord and Stamps 2009; Peiman and Robinson 2010). When ecologically similar species come into secondary contact, agonistic interactions emerging from interference competition can cause selection on traits that affect fighting ability, encounter rates, or competitor recognition (Grether et al. 2009). Such agonistic character displacement (ACD; Grether et al. 2009) effectively reduces interspecific interference competition and may facilitate competitive exclusion of subordinate taxa, thereby influencing community structure (Miller 1964; Bovbjerg 1970; Gill 1974; Murray 1981; Peiman and Robinson 2010).

Agonistic character displacement often involves divergence of signals used in competitor recognition (Grether et al. 2009). For example, *Hetaerina* damselfly wing coloration spots involved in territorial aggression exhibit shifts in sympatry to minimize interspecific fighting (Anderson and Grether 2010). Alternatively, ACD may involve shifts in receiver responses to agonistic signals. Male poison dart frogs (*Allobates*) in sympatry with heterospecifics that emit aggressive calls at similar frequencies exhibit increased discriminative ability compared to allopatric populations, reflecting release from competitive masking interference (Amézquita et al. 2006). However, little is known about the proximate mechanisms mediating competitor recognition systems (Grether 2011). Elucidating the neural mechanisms underlying ACD can provide important insight into the genetic basis and/or phenotypic plasticity involved in interspecific recognition (ten Cate and Rowe 2007; Grether et al. 2009).

Among animals that use acoustic signals to repel rivals and attract mates, the peripheral auditory system is often tuned to match the frequencies contained in conspecific vocalizations (Capranica 1965; Capranica and Moffat 1983; Ryan and Keddy-Hector 1992; Riabinina et al. 2011). Efficient encoding of conspecific signals is also facilitated by tonotopic neurons in the auditory cortex, inferior colliculus, and medial geniculate (Ehret and Giessler 2005; Malmierca et al. 2008). Sympatric species shift the tuning of their auditory systems to reduce responses to heterospecific signals, a phenomenon known as a “sensory filter” (Capranica and Moffat 1983). Such filtering occurs in intersexual contexts to reduce the costs of interspecific mating. In principle, however, animals may preserve the function of peripheral sensory systems but assign species-specific stimuli different motivational values, a phenomenon we refer to as a “motivational filter”. The latter scenario seems more plausible when heterospecifics are ecologically salient and represent a potential threat.

Neotropical singing mice are diurnal insectivorous rodents that inhabit montane cloud forests throughout Central America. Alston’s singing mouse (*S. teguina*; 9-12 g) is a widespread species inhabiting mid- to high-altitude cloud forest, forest edge, and abandoned pastures from southeastern México to western Panamá (1000-2900 m), whereas the Chiriquí singing mouse (*S. xerampelinus*; 13-15 g) is restricted to the highest forested summits and páramo of Costa Rica and Panamá (2200-3300m; Wilson and Reeder 2005; Hooper and Carleton 1976). Males of both species commonly emit rapid frequency-modulated trills that function in male-male aggression and female mate choice. Vocalizations of *S. teguina* are long (4-7 s), decelerating trills of moderate power (54 dB SPL at 1 m) and high dominant frequencies (22-26 kHz), whereas *S.*

xerampelinus trills are shorter (1-2 s), louder (63 dB SPL at 1 m), and lower in frequency (16-20 kHz: Campbell et al. 2010). On the highest peaks of Costa Rica and Panamá, *S. teguina* is abruptly replaced and aggressively excluded from higher elevations by the larger, behaviorally dominant *S. xerampelinus* (Chapter 4). In a large-scale analysis of geographic variation in songs, we found no character displacement in the structure of trills between sympatric and allopatric populations of *S. teguina* (Campbell et al. 2010). However, the behaviorally subordinate *S. teguina* reduces song rate when broadcast trills of *S. xerampelinus*, suggesting an ability to distinguish between conspecifics and heterospecifics in sympatry (Chapter 4).

In this study, we tested for ACD in song recognition by investigating auditory perception of *S. teguina*. We performed playback studies in the lab using male *S. teguina* from a population that co-occurred with *S. xerampelinus* (sympatric) and those that did not (allopatric). Induction of the immediate early gene *egr-1* was quantified in the auditory cortex and medial geniculate to identify mechanisms of vocal recognition. We predicted that the auditory system would increase its sensitivity to heterospecifics in sympatry to accommodate the ecological salience of the stimulus.

Materials and Methods

Reciprocal Playback Experiments

We live-captured animals in Sherman traps baited with peanut butter and oats on Volcán Irazú (VI- sympatry) and Cerros La Carpintera (LC-allopatry), Cartago Province, Costa Rica (Figure 5-1). Adult males were housed singly in 28 x 28 x 28 cm PVC-coated wiremesh cages and isolated in anechoic chambers for 24 hrs prior to initiating laboratory playback trials between 0600-1000. Individuals were assigned randomly to playback of 1 of 3 stimuli: Control stimuli, heterospecific song, or conspecific song ($n = 8$

per treatment at each site). We selected 3 songs from each of 4 individuals of each species from laboratory recordings of the respective focal population (Campbell et al. 2010). Control stimuli were synthesized by generating white noise bounded within species-specific frequency envelopes (10-42 kHz). At LC, heterospecific stimuli were selected from *S. xerampelinus* recorded at VI. We played stimuli at 50 dB SPL at 1 m from one of four randomly selected Pioneer TS-250 speakers placed on each side of the mesh cage. Mice were recorded for 20 min to obtain pre-stimulus song rate. At 20, 21, and 22 min, we broadcast each of 1 song followed by 2 minutes of silence. The sequence was repeated three times with songs from different males. For control treatments, the single control stimulus was broadcast at the same rate as conspecific and heterospecific song treatments. We recorded the total number of songs emitted by focal animals in the 20 min period following the initial stimulus. We also calculated the amount of time animals spent on the half side of the cage that held the speaker emitting the stimulus.

Tissue Preparation

From a subset of individuals (n = 4 per treatment per population), we extracted brains from euthanized animals 60 min after broadcast of the final stimulus. Brains were drop-fixed in 4% paraformaldehyde in phosphate buffered solution (PBS) and stored at 4°C for 14 d at each field site. Brains were then cryoprotected for 24-48 hrs in 30% sucrose made in 4% paraformaldehyde-PBS and stored at -80°C until sectioning. Coronal sections from the olfactory bulbs through the cerebellum were cut in 40 µm sections on a cryostat (Leica 1900, Leica, Microsystems, Heidelberg, Germany). Sections were collected into 24-well plates containing a cryoprotectant solution (PBS containing 30% sucrose, 30% ethylene glycol, and 10% polyvinylpyrrolidone) and

stored at -20°C until processing for *egr-1* immunoreactivity.

Egr-1 Immunocytochemistry

Floating sections were washed in PBS, incubated for 15 minutes in 3% H₂O₂ in 0.01 M PBS (pH 7.2–7.4) to quench endogenous peroxidase activity, washed in PBS, blocked for 1 hour in 10% normal goat serum (NGS) with 3% Triton-X in PBS (PBSTX), and washed in PBS. Sections were then incubated overnight at 4°C with rabbit polyclonal antibody directed against *egr-1* (Santa Cruz Biotechnologies, sc-189), diluted 1:250 in 5% NGSPBSTX. Control sections were incubated without the primary antibody. The next day sections were washed in PBS, incubated for 1 hour with biotinylated goat antirabbit IgG, rinsed in PBS, and incubated for 1 hour in avidin-biotin-horseradish peroxidase solution (Leinco, St. Louis, MO; R106 and A106, both diluted 1:200 in 5% NGS-PBSTX). We used the peroxidase/diaminobenzidine (DAB) method with nickel intensification to visualize *egr-1* protein. Sections were immersed in 0.07% DAB (Sigma, St. Louis, MO) in PBS with 0.1% nickel chloride and 0.03% H₂O₂, and developed for 2 minutes until antibody-antigen binding sites were stained black. The reaction was stopped in PBS. Rinsed sections were mounted onto slides, air-dried overnight, dehydrated in serial alcohol dilutions, and coverslipped from xylene with Eukitt (Calibrated Instruments, Hawthorne, NY).

Analysis of Egr-1 Immunoreactivity

The auditory cortex and medial geniculate were identified using landmarks of *Scotinomys* (Campbell et al. 2009), supported by the mouse and rat atlases (Paxinos and Watson, 1998; Paxinos and Franklin, 2001; approx. Bregma – 3.16 mm). We selected at random 2–3 alternate sections per region. Projected images of sections were visualized at 20X using an Axiocam Zeiss light microscope mounted to a CCD

camera (Leica DFC 310FX) and interfaced with a computer. Automatic adjustment of light intensity normalized background levels. A 350 x 350 μm box was placed on digitized sections for each individual and images were constructed in Adobe Illustrator CS3 (San Jose, CA, v. 13.0.0). A researcher blind to experimental conditions quantified the number of *egr-1* stained-nuclei.

Statistical Analyses

We used the Scheirer–Ray–Hare technique (Sokal and Rohlf 1995) to test for significance of playback treatment on response (post – pre stimulus) between sympatric and allopatric populations of *S. teguina*. Analysis of variance was used to assess differences in time spent near speakers broadcasting the stimuli and to test for differences among treatment groups in *egr-1* induction. We conducted post-hoc comparisons using Tukey’s test. Averages are reported \pm SE.

Results

Response of *S. teguina* to heterospecific stimuli depended upon whether they were sympatric or allopatric with *S. xerampelinus* (Treatment x population interaction, $H = 12.36$, $P < 0.002$). *S. teguina* decreased singing after heterospecific playback in sympatry (Pre: 2.13 ± 0.35 ; Post: 1.0 ± 0.4 songs) but increased singing in allopatry (Pre: 0.38 ± 0.18 songs; Post 1.38 ± 0.26 songs; $P < 0.05$; Figure 5-2). Similarly, animals spent less time near heterospecific stimuli in sympatry (Control: $43.8 \pm 11.2\%$; Conspecific: $73.3 \pm 5.8\%$; Heterospecific: $11.8 \pm 6.0\%$) but spent similar amounts of time near conspecific and heterospecific stimuli in allopatry compared to controls (Control: $33.1 \pm 6.9\%$; Conspecific: $64.7 \pm 9.2\%$; Heterospecific: $56.2 \pm 6.6\%$; $P < 0.05$; Figure 5-2).

Both populations exhibited significant induction of *egr-1* in response to conspecific songs when compared to control stimuli. Surprisingly, heterospecific songs elicited stronger responses in sympatry than in allopatry, despite the fact that these songs elicit less singing behavior in sympatry (Figure 5-3).

Discussion

We found that allopatric *S. teguina* responded to heterospecifics, whereas sympatric animals decreased singing. Behavioral responses were reflected in neural auditory tuning. Such agonistic character displacement in social cognition reflects emancipation from interspecific aggression in sympatry.

Our data suggest that the auditory system can increase its response to heterospecific vocalizations in ecologically relevant contexts. Recent work demonstrates that both reward and punishment can increase mammalian auditory responses to sounds associated with either positive or negative outcomes (e.g. Resnik et al. 2011). We suggest that differences in aggressive experience with heterospecifics can modify auditory tuning to match ecologically important stimuli. Results from reciprocal transplant experiments, where allopatric *S. teguina* experience heterospecific aggression paired to *S. xerampelinus* song and sympatric *S. teguina* are removed from interspecific aggression, will help elucidate the role of learning in shaping brain and behavioral responses.

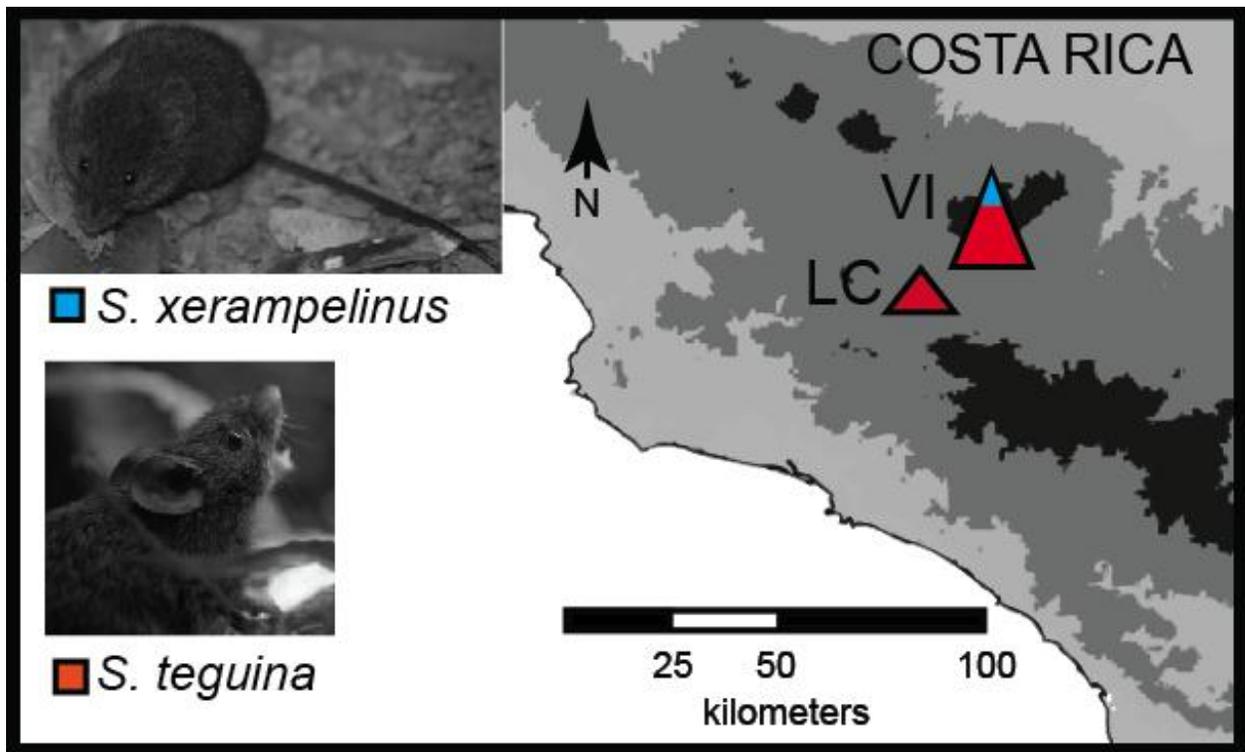


Figure 5-1. Altitudinal distributions of Neotropical singing mice (*Scotinomys*) at our study site in Costa Rica. *S. xerampelinus* (blue) replaces *S. teguina* (red) on Volcán Irazú (VI). *S. teguina* is allopatric on Cerros La Carpintera (LC).

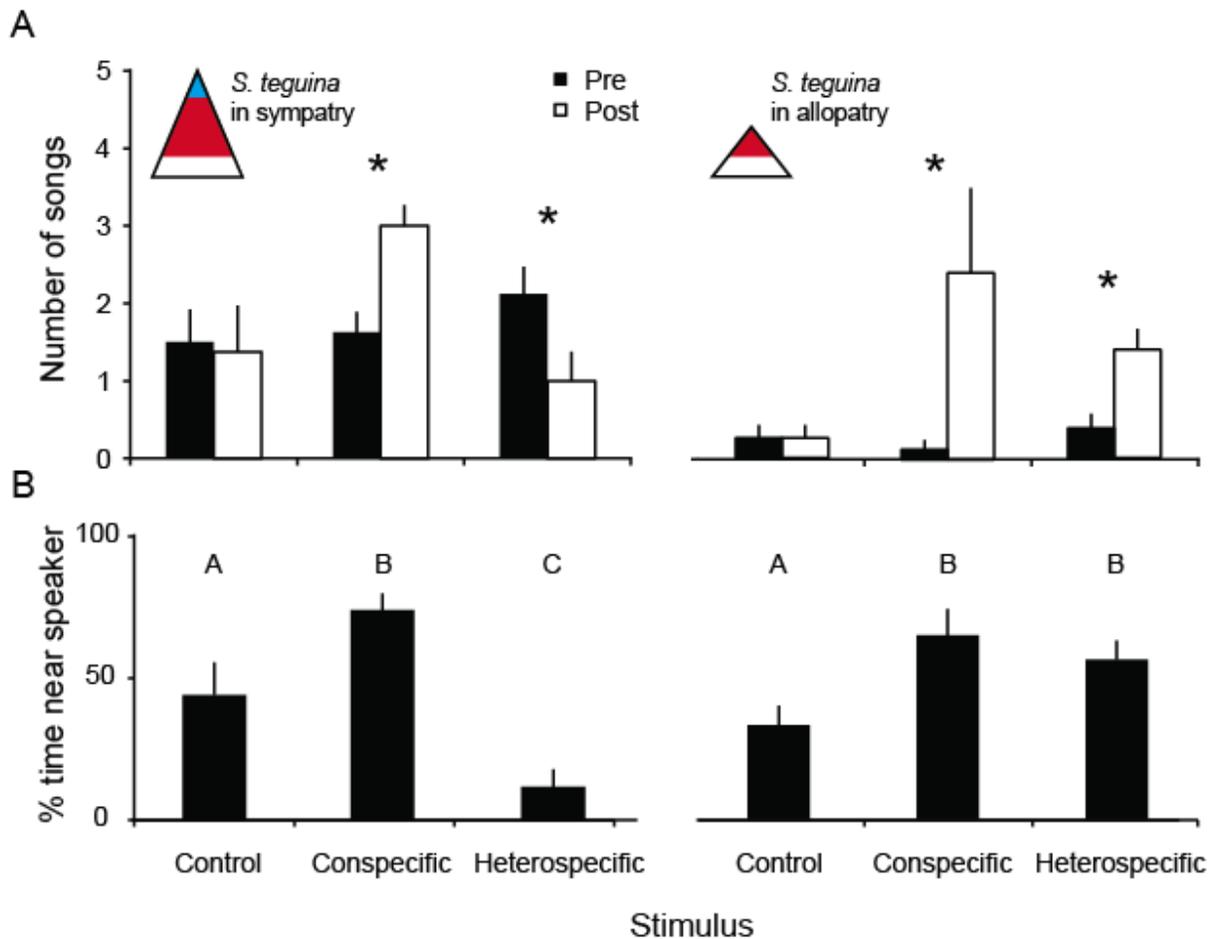


Figure 5-2. Response to playback trials in the laboratory at Volcán Irazú, Costa Rica (sympatry) and Cerros La Carpintera (allopatry). A) Number of songs produced by *S. teguina* pre- (shaded bars) and post- (open bars) playback of control, conspecific, and heterospecific stimuli. B) Percent time spent near speakers broadcasting sound stimuli. Error bars are ± 1 s.e. $*P < 0.05$. Letters not sharing the same letter are significantly different. $P < 0.05$.

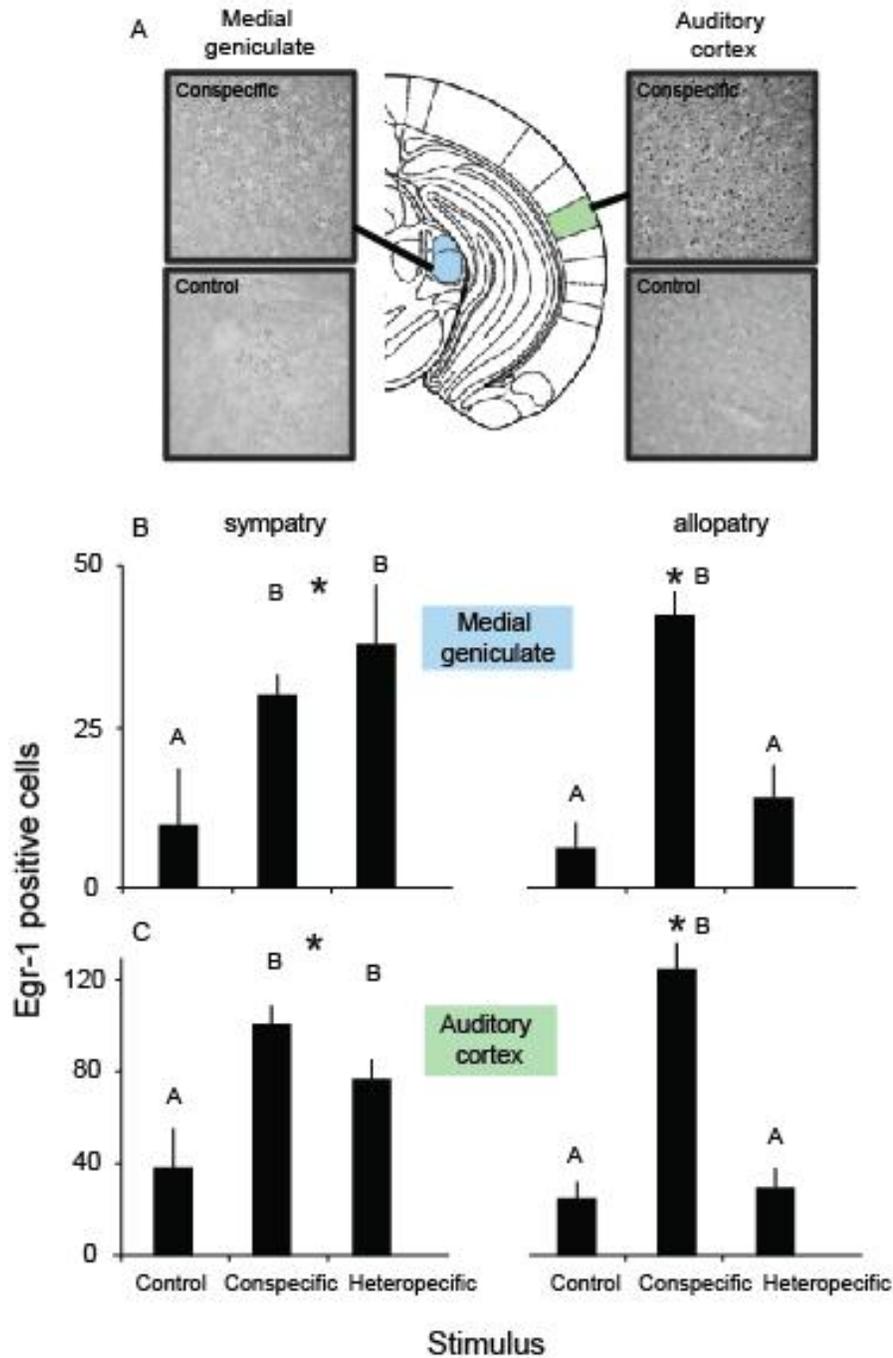


Figure 5-3. Neuronal immediate early gene expression in response to playbacks in the laboratory at Volcán Irazú (sympatry) and Cerros La Carpintera (allopatry), Costa Rica. A) Brain regions and representative Egr-1 expression in response to playback of stimuli. B) Egr-1 expression in the medial geniculate. C) Egr-1 expression in the auditory cortex. Error bars are ± 1 s.e. Letters not sharing the same letter are significantly different.

CHAPTER 6 CONCLUSIONS

My dissertation addressed proximate and ultimate factors mediating acoustic communication in Neotropical singing mice (*Scotinomys*) with the goal of understanding how biotic and abiotic factors limit their altitudinal distributions. The work incorporated biogeographical surveys, long-term environmental monitoring, reciprocal removal experiments, behavioral trials, playback experiments in the field and laboratory, hormonal manipulation, and analysis of neuronal immediate early gene expression. The integrative nature of the study enabled me to link changes in perception at the neural level to changes in distribution at the geographic level. I highlight my findings below.

Androgens are an important class of steroid hormones involved in modulating the expression and evolution of male secondary sex characters. Vocalizations used in the context of aggression and mate attraction are among the most elaborate and diverse androgen-dependent animal displays as reflected in a rich tradition of studies on bird song and anuran calls. Male Alston's singing mice (*S. teguina*) commonly emit trilled songs that appear to function in male-male aggression. In this study, we experimentally manipulated androgens in singing mice to assess their role in modulating aggression and song effort. Testosterone- and DHT-treated animals retained aggressive and song attributes similarly. However, castrated mice administered empty implants showed more subordinate behavior and sang fewer songs that were shorter, lower in power, higher in frequency, and less stereotyped. The extensive effects of androgens on a suite of phenotypes highlight their role in linking gonadal status with decisions about investment in reproductive behaviors.

Vocalizations used in aggressive and mating contexts often convey reliable information about signaler condition when physical or physiological limitations constrain signal expression. In vertebrates, androgens modulate the expression of vocal signals and provide a proximate link between male condition and signal form. In many songbirds, assessment of males is based on production of trills that are constrained by a performance trade-off between how fast notes are repeated and the frequency bandwidth of each note. In this study, we recorded trills of male Neotropical singing mice to ask if they exhibited a similar performance trade-off, and then manipulated androgen levels to assess their role in modulating vocal performance. Lastly, we broadcast experimentally manipulated trills to females to determine if they preferred versions resembling those of androgen-treated males. Singing mice exhibit a vocal performance trade-off similar to birds. Males treated with androgens maintained vocal performance, but castrated mice administered empty implants produced trills with lower performance. Females approached high performance trills more rapidly and spent more time near corresponding speakers. Together, our results demonstrate that androgens modulate the production of physically challenging vocalizations, and the resulting signal variation influences female receiver response.

Interspecific aggression between ecologically similar species may influence geographic limits by mediating competitive exclusion at the range edge. Within species, male advertisement vocalizations mediate competitive and dominance interactions. Between species, vocalizations may likewise contribute to dominance interactions and influence species distributions. We studied the mechanisms underlying altitudinal range limits in Neotropical singing mice, a genus of muroid rodent in which males vocalize to

repel rivals and attract mates. We first delineated replacement zones and described temperature regimes on three mountains in Costa Rica and Panamá where Chiriquí singing mice (*S. xerampelinus*) abruptly replace Alston's singing mice (*S. teguina*). Next, we conducted interspecific behavioral trials and reciprocal removal experiments to test whether interspecific aggression mediated species replacement. Finally, we performed reciprocal playback experiments to investigate whether response to song matched competitive interactions. Biogeographical surveys suggest that *S. xerampelinus* may be limited by temperature tolerance. Behavioral trials and removal experiments indicate that *S. xerampelinus* is behaviorally dominant and excludes *S. teguina* from higher altitudes. Playback experiments demonstrated that *S. teguina* is silenced and repelled by heterospecific song, whereas *S. xerampelinus* responded to heterospecifics. Thus, response to song reflects underlying dominance and suggests that interspecific communication mediates altitudinal zonation of ecologically similar congeners.

Species recognition touches on deep themes in diverse domains, ranging from the origin of species to the nature of social cognition. While evolutionary and ecological aspects of species recognition have received much attention, the neural substrates underlying receiver decisions are far less explored. When related species occur in sympatry, receiver responses to heterospecific signals are often altered in comparison to allopatric populations. Such character displacement in communication presents an opportunity to explore ecological variation in the mechanisms of social cognition. Among animals that use acoustic signals to identify conspecifics, such as insects and anurans, sympatric species often shift the tuning of their auditory systems to reduce responses to

heterospecific signals, a phenomenon known as a “sensory filter”. In principle, it is possible that animals could preserve the function of peripheral sensory systems, but assign species-specific stimuli different motivational values, a phenomenon we refer to as a “motivational filter”. We tested these alternatives in two populations of Alston’s singing mouse (*Scotinomys teguina*). Neotropical singing mice are diurnal insectivorous rodents that inhabit montane cloud forests throughout Central America. Males commonly emit rapid frequency-modulated trills that function in male-male aggression and female mate choice. At some sites, *S. teguina* co-occurs with the larger, behaviorally dominant Chiriqui singing mouse (*S. xerampelinus*). We performed playback studies in the lab and field using male *S. teguina* from populations that co-occurred with *S. xerampelinus* (sympatric) and those that did not (allopatric). We found that allopatric male *S. teguina* sang back in response to both *S. teguina* and *S. xerampelinus* songs. In contrast, male *S. teguina* from populations sympatric with dominant *S. xerampelinus* seem to suppress song in response to heterospecific playback. To examine whether species-specific responses were modulated by the tuning of auditory system, we examined song-evoked induction of *egr-1*, an immediate early gene often used in the study of bird song, in the medial geniculate and auditory cortex of *S. teguina* males. Both populations exhibited significant induction of *egr-1* in response to conspecific songs when compared to control stimuli. Surprisingly, we found that heterospecific songs elicited stronger responses in sympatry than in allopatry, despite the fact that these songs elicit less singing behavior in sympatry. These data refute the sensory filter hypothesis for this species, and suggest that the auditory system increases its response to heterospecific vocalizations. Recent

work demonstrates that both reward and punishment can increase mammalian auditory responses to sounds associated with either positive or negative outcomes. We suggest that differences in aggressive experience with heterospecifics can modify auditory tuning to match ecologically important stimuli.

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BIOGRAPHICAL SKETCH

Bret was born in Point Pleasant, New Jersey and grew up in nearby Whiting on the northern terminus of the Pine Barrens. His exploration of the fauna inhabiting the forest, streams, and bogs served as initial inspiration for his work in biology. He attended Manchester Township High School before enrolling as a biology major at Ursinus College, Collegeville, PA. Two courses made a significant impact on his development: *Biology of tropical rainforests in Costa Rica* brought him to the tropics for the first time, and *Mammalogy* served to formalize his interest in the study of organismal biology. Under the mentorship of Dr. Ellen Dawley, Bret conducted independent research on salamanders and tree squirrels-the latter leading him to pursue his Master's degree with Dr. John Koprowski at the University of Arizona in Tucson. Here he spent many days exploring the sky islands of the desert southwest, with most time in pursuit of Apache fox squirrels in the Chiricahua Mountains. His time in Arizona was capped off by enrollment in a flagship course in tropical biology through the Organization for Tropical Studies, where he first captured and recorded Neotropical singing mice in the Talamancas of Costa Rica. He went on to enroll in the Department of Biology at the University of Florida and spent most of his time in the mountains of Panamá and Costa Rica studying the biology of Neotropical singing mice. Bret currently resides in Gainesville, FL with his wife Kate, his daughter Mary, and their two blue dogs, Arlo and Badger.