

## **Project Summary**

Understanding the role of abiotic and biotic factors in determining species distributions is a fundamental goal in biology. In many organisms, physiological tolerance to temperature and interspecific competition interact to delimit the range edge. Although the molecular basis of temperature tolerance is known, proximate mechanisms that mediate interspecific competition remain unexplored. How do species perceive and subsequently respond to heterospecific stimuli? What are the neural substrates that contribute to species recognition? In the proposed study, we explore how behavioral and neural mechanisms moderate competitive interactions between species and, in turn, limit species distributions. We use a novel mammalian model, the singing mouse, to address a long-standing topic in ecology from an ethological and evolutionary perspective.

## **Intellectual Merit**

The integrative nature of the study enables us to link changes in perception at the neural level to changes in distribution at the geographic level. Examining the mechanisms and function of mammalian song presents exciting opportunities to identify convergent and divergent pathways of perception in birds and other vertebrate taxa. From an applied perspective, insight into the role of interspecific competition in limiting species distributions has direct application to atmospheric warming scenarios. Rising temperatures are predicted to force montane species upward to eventual extinction. Resistance to this 'escalator effect' will depend in part on a species competitive ability (Marris 2007). Thus, incorporating species interactions into climate models will improve our ability to identify taxa most vulnerable to global warming.

## **Broader Impacts**

*International Networks & Outreach-* I am committed to providing research opportunities to underrepresented groups in science. During my master's research, I recruited and provided training to 5 undergraduate students (including 2 U.S. women and 1 Columbian male), all of whom went on to enroll in graduate programs in biology. I recently sponsored a Panamanian colleague's membership to the American Society of Mammalogists and continue to seek opportunities to collaborate with Latin American biologists. Last summer, I recruited a Costa Rican undergraduate and a local farmer to assist in data collection and presentation. I also conducted public education and outreach in the local communities where I work. Here, I emphasized hypothesis-driven inquiry into the function of animal behavior and underscored the importance of cloud forest conservation.

*Local Involvement & Education-* As a member of the University of Florida community, I am similarly committed to the involvement and education of underrepresented groups. Last year, I mentored a female undergraduate who is now applying to graduate school. I am currently mentoring an African-American undergraduate who was recently granted a McNair Fellowship from the US Department of Education. The McNair scholars program prepares underrepresented undergraduate students in science for pursuit of a doctoral degree by providing money, mentoring, and research experience. He and I are conducting an androgen implant study to understand the hormonal control of song in singing mice. I am also working with my committee member, Dr. Doug Levey, to develop an interactive module on animal behavior in his NSF-funded SPICE (Science Partners in Inquiry-based Collaborative Education) program. I will bring in mice from our lab colony at the University of Florida to middle school classrooms with high minority enrollments and incorporate audio and video from my playback experiments to discuss alternative hypothesis-testing and links between the ecosystem, organism, and brain.

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## Behavioral and neural mechanisms of altitudinal replacement in singing mice

### INTRODUCTION

Understanding the role of abiotic and biotic factors in determining species distributions is a fundamental goal in biology. In many organisms, physiological tolerance to temperature and interspecific competition interact to delimit the range edge (Connell 1981, Schoener 1983, Case and Taper 2000). Although the molecular basis of temperature tolerance is known (Pörtner et al. 2002, Pörtner and Knust 2007), proximate mechanisms that mediate interspecific competition remain unexplored. How do species perceive and respond to heterospecific stimuli? What are the neural substrates that contribute to species recognition? In the proposed study, I explore how behavioral and neural mechanisms moderate competitive interactions between species and, in turn, limit species distributions.

Rodents have long been a model system for the study of interspecific competition (Munger and Brown 1981, Brown and Munger 1985). Consistent evidence from removal experiments and observational studies shows that interspecific competition influences the diversity and composition of rodent assemblages over short and long time scales (Heske et al. 1994). Yet, little is known about how behavioral mechanisms modulate species interactions, mostly due to difficulties in observing small mammals in nature. We take advantage of song, a unique phenotype in rodents, to study interspecific interactions between mice in the genus *Scotinomys*.

Singing mice (*Scotinomys*) inhabit Neotropical mountains and are remarkable for their audible advertisement songs used in male-male interactions (Pasch, below; Fig. 1). The two species of mice, *S. teguina* and *S. xerampelinus*, are similar ecologically and come into contact in the highlands of Costa Rica and Panamá. Here, *S. teguina* is subordinate to *S. xerampelinus* (Pasch, below) and may be prevented from colonizing higher altitudes by the presence of its congeneric competitor. The system presents a unique opportunity to explore how song mediates interspecific aggression and limits species distributions. Furthermore, foundational work on neural circuits of aggression and fear in laboratory rodents provide a predictive framework to assess species-specific responses to conspecific and heterospecific song. The central goal of my study is to examine how interspecific competition influences behavioral and neural mechanisms of song perception. I propose two specific aims:



Fig. 1. Singing male *S. teguina*.

#### AIM I. Neural mechanisms of song perception in sympatry

Identify brain regions in *S. teguina* and *S. xerampelinus* that mediate perception of conspecific and heterospecific song.

#### AIM II. Behavioral response and neural mechanisms of song perception in allopatry

Determine behavioral response and identify brain regions in *S. teguina* that mediate perception of conspecific and heterospecific song.

### BACKGROUND

*Natural history of singing mice*- Singing mice are diurnal insectivorous rodents distributed throughout cloud forests of Central America. Alston's singing mouse (*S. teguina*) inhabits mid- to high-altitude forests and pastures from southeastern México to western Panamá, whereas the Chiriqui singing mouse (*S. xerampelinus*) is restricted to the highest summits of Costa Rica and Panamá (Wilson and Reeder 2005; Fig.1). The two species show considerable overlap in prey items (Hooper and Carleton 1976) and isotopic

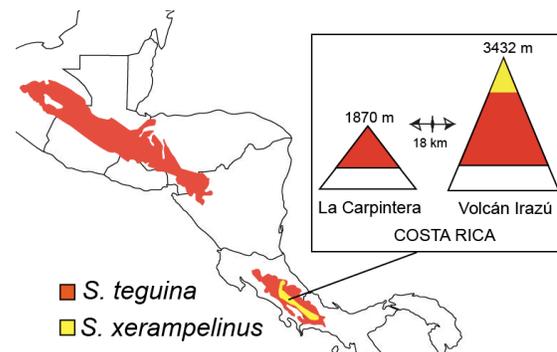


Fig. 2. Geographic distribution of singing mice and altitudinal distribution at proposed study sites.

signatures ( $\delta_{15}\text{N}$ ,  $\delta_{13}\text{C}$ ) of diet in sympatry (Pasch, unpublished data). Both species emit a stereotyped trill consisting of pulse train of frequency sweeps (10-50 kHz; Miller and Engstrom 2007; Fig. 2). Songs of *S. teguina* are longer (6-12 s) and have a higher dominant frequency (26 kHz) than songs of *S. xerampelinus* (2-4 s, 18 kHz), thus encoding information about species identification over a distance of ~25 m (Pasch, unpublished data).

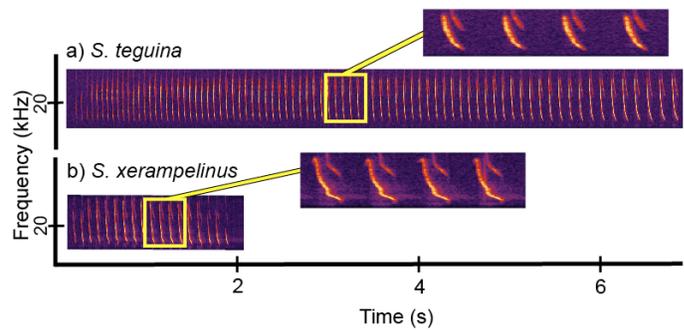


Fig. 3. Songs of a) *S. teguina* and b) *S. xerampelinus*

Males are more aggressive and sing more than females (Hooper and Carleton 1976), and the song appears to function in male-male aggression (Pasch, below).

*Song and neural mechanisms of recognition-* Organisms that use acoustic stimuli to communicate are ideal models to identify the behavioral and molecular underpinnings of competitive interactions. In birds, interspecific territoriality is enforced by song (Catchpole and Leisler 1986, Robinson and Terborgh 1995). Subordinate species retreat from dominant species' song, whereas dominant species aggressively contest subordinate species' song (Martin and Martin 2001). Specific regions of the "social behavior network," (Newman 1999, Goodson 2005) including the lateral septum, medial and central amygdala, and anterior hypothalamus, are induced in response to aggressive challenges (Goodson et al. 2005) and provide a predictive framework to assess species-specific responses to conspecific and heterospecific song. Although neural circuits of aggression and fear are well-characterized in vertebrates (Davis 1992, LeDoux 2000, Nelson and Trainor 2007), few studies have explored how such regions act to translate stimulus recognition into behavioral action in an ecological context (Heimovics and Ritters 2005). As markers of neuronal activity, immediate early genes provide a powerful method of identifying brain regions that respond to particular stimuli (Clayton 2000).

## RESEARCH PLAN

The broad goal of my dissertation is to understand how abiotic and biotic factors limit the altitudinal distributions of singing mice. In the proposed study, I focus on biotic factors to understand how behavioral and neural mechanisms moderate competitive interactions. Below, I briefly describe results from preliminary studies on both abiotic and biotic components to frame the study in a broader context and provide a conceptual foundation for my proposed aims.

### Preliminary Data

*A. Variation in altitudinal limits and temperature regimes* - In summer 2007, I established trapping transects to demarcate zones of altitudinal replacement on 3 mountain ranges in Costa Rica and Panamá. I placed temperature loggers in the understory where both species were captured to collect ambient temperature every hour for 2 years. We found that *S. xerampelinus* abruptly replaces *S. teguina* over a linear distance of < 300 m, concomitant with a change in vegetation from successional scrubland/pasture to mature forest. The altitude of replacement differs among mountains (Fig. 4a), yet temperature regimes are strikingly similar (ANOVA,  $P > 0.01$ ;  $\bar{x} = 10.5 \pm 0.8$  °C; Fig. 4b).

*B. Reciprocal removal experiment* - In summer 2008, I returned to Volcán Irazú to perform a reciprocal removal experiment. I established twelve 32 m x 24 m plots of 20 traps spaced 8 m apart in a rectangular grid. I paired control and removal plots for a given species (*S. teguina* or *S. xerampelinus*) and had 3 replicates per species. Pairs of control-removal plots were spaced 100 m apart and were separated from other paired plots by > 500 m. I placed temperature loggers in the center of all plots and generated random points to quantify understory density, canopy cover, and number of trees.

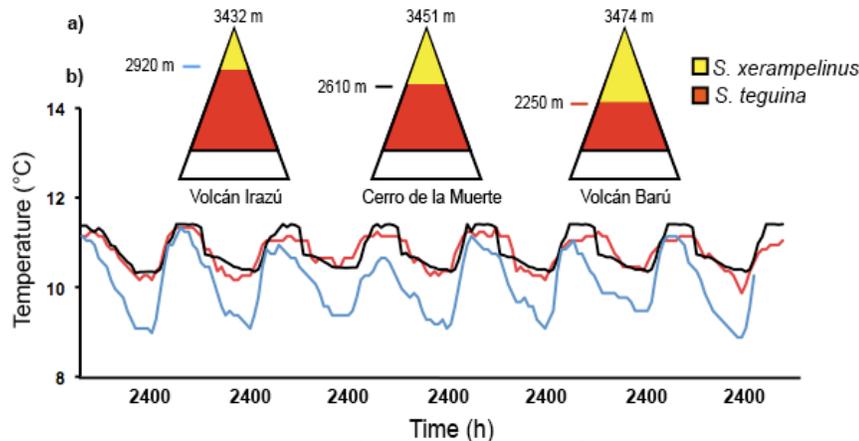


Fig. 4. a) Altitudinal replacement zones of *S. xerampelinus* over *S. teguina* on 3 mountain ranges in Costa Rica (Irazú and Muerte) and Panamá (Barú). b) Daily temperature regimes in zones of altitudinal replacement at Irazú (2920 m), Cerro de la Muerte (2610 m), and Barú (2250 m). Note: Temperatures plotted for 1 week, although data collection is ongoing for 2 years.

There were fewer trees (*t*-test,  $P < 0.001$ ), less canopy cover (*t*-test,  $P < 0.001$ ), and more understory density (*t*-test,  $P < 0.05$ ) on *S. teguina* plots. Vegetation characteristics were reflected in temperature profiles, with *S. teguina* plots having higher daily averages (*t*-test,  $P < 0.001$ ) and greater variation (*t*-test,  $P < 0.001$ ; Fig. 5). I trapped for 26,880 trap-hours over 9 weeks and captured 121 *S. teguina* (removed 65) and 71 *S. xerampelinus* (removed 34). After controlling for natural recruitment rates on control plots, I found a significant asymmetrical response to removals ( $P < 0.05$ ). *S. teguina* ( $n = 19$ ) began to colonize *S. xerampelinus* removal plots within 3 weeks and remained there for the duration of the study. Conversely, I only captured 1 male *S. xerampelinus* in a *S. teguina* removal plot one time.

#### C. Behavioral interactions in arenas

In summer 2007, I staged heterospecific interactions between adult males captured in sympatry at Cerro de la Muerte and Volcán Irazú. Animals were placed in an arena separated by an opaque partition for 2 d after capture. I then removed the partition and allowed animals to interact for 5 min. I videotaped all trials and scored number of aggressive chases and attacks. In every trial, *S. xerampelinus* exhibited more chases and attacks (Binomial exact,  $P < 0.005$ ,  $n = 12$ ; see video at [www.zoology.ufl.edu/bpasch/Site/research.html](http://www.zoology.ufl.edu/bpasch/Site/research.html)).

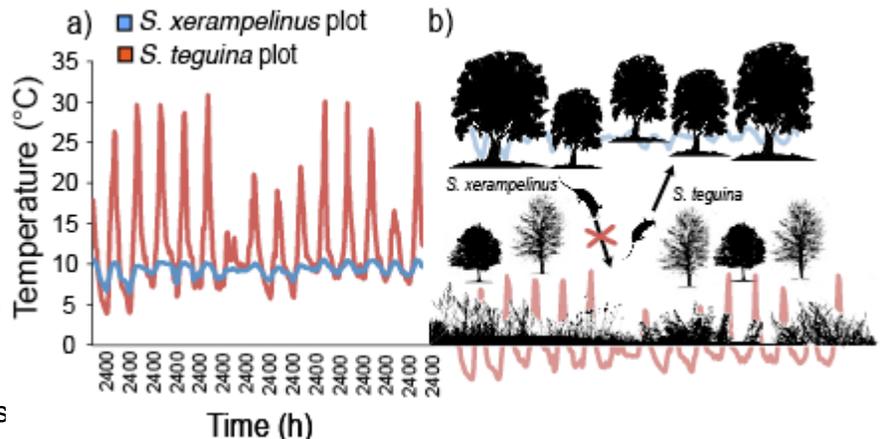


Fig. 5. a) Representative temperature profiles of *S. xerampelinus* and *S. teguina* plots. b) Diagram of vegetation characteristics of plots and response to reciprocal removal experiment.

**D. Field playback experiments in sympatry** - As part of my reciprocal removal experiment, I identified resident male home ranges based on recapture data from control plots. I visited sites between 6am -11am and waited 10 min before initiating playbacks. Songs used in playback experiments were recorded from males of the same population during the previous summer. I selected 2 representative songs from 8 males of each species and generated band-pass filtered noise (WHITE) to match spectral envelopes of each call. Three stimuli types were played at each trial: Conspecific (CON), Heterospecific (HET), and a control noise (WHITE). I matched duration and root mean square (RMS) amplitude of WHITE to CON. I randomized the order of HET and WHITE and always played CON songs last to avoid carryover in response to

subsequent stimuli (Martin and Martin 2001; see below). After a 5 min prestimulus period, I played 2 stimuli (1 / min) and allowed 5 min before playing subsequent stimuli. I used a reference tone and sound level meter to calibrate amplitude of playback to match average *S. teguina* (54 dB at 1m) or *S. xerampelinus* (63 dB at 1 m) songs. We recorded all trials with a Sony PCM-50 digital recorder and determined species identity of evoked songs using discriminant function scores based our existing call library. Generalized linear models (GLM) with Poisson distributions were used to analyze responses.

Both species respond to conspecific calls (Fig. 6). We found a significant interaction between HET and target species (GLM, HET \* *S. teguina*,  $P < 0.005$ ), indicating that *S. xerampelinus* sings in response to *S. teguina* (Fig. 6a), but *S. teguina* does not sing in response to *S. xerampelinus* (Fig.6b). Playback order did not influence response of either species (GLM, order effect,  $P > 0.10$ ).

**E. Lab playback experiments in sympatry** - I used adult males captured on removal plots to perform playback experiments in a controlled setting. Twenty-four adult males of each species were assigned randomly to 1 of 3 playback treatments: CON, HET, or WHITE (white noise matched to the amplitude and duration of CON). Animals were isolated in soundproof chambers for 24 hrs after capture. I selected songs from 4 individuals of each species with 3 recordings. I generated white noise bounded within species-specific frequency envelopes (*S. teguina* 10-42 kHz, *S. xerampelinus* 10-38 kHz) and applied a -20 dB linear fade to the beginning of white noise stimuli to approximate the amplitude envelope of a natural call. We started videotaping mice 20 minutes prior to first stimulus (Pre). We played 1 stimulus/minute for 3 minutes followed by 2 minutes of silence and repeated the sequence three times (“During” = 20 min). Animals were euthanized 60 min after the last stimulus (Post) and brains were fixed in 4% paraformaldehyde. I used a repeated measures ANOVA with time (pre, during, post) as a within-subject factor and treatment (CON, HET, WHITE) as between-subject factors. Results mirror field playback experiments, where *S. xerampelinus* sings in response to *S. teguina*, but *S. teguina* does not sing in response to *S. xerampelinus* (Fig. 7). Carryover was also noticeable after CON stimuli, as both *S. teguina* and *S. xerampelinus* maintain higher song rates in POST.

**Inferences and Directions** - Results from removal experiments match asymmetrical temperature tolerances of *S. teguina* and *S. xerampelinus*. Hill and Hooper (1967) found that *S. teguina* is able to survive at colder ambient temperatures, whereas *S. xerampelinus* dies when exposed experimentally to warm ambient temperatures. Our data suggest that *S. xerampelinus* is

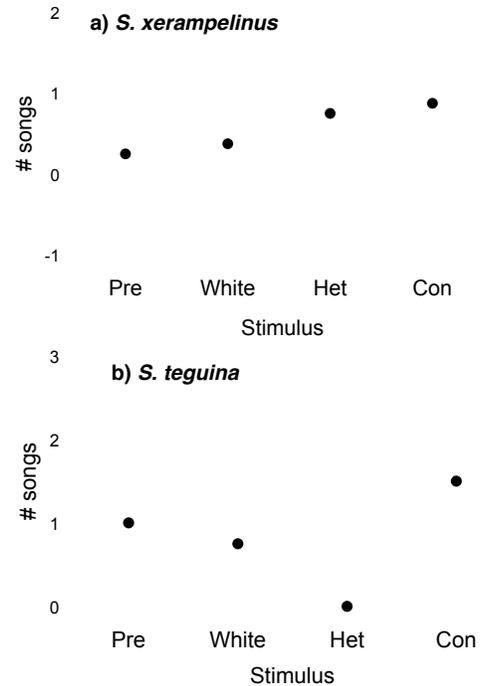


Fig. 6. Response of a) *S. xerampelinus* (n = 8) and b) *S. teguina* (n = 8) to playback stimuli in the field. PRE- prestimulus, White-control, Het-heterospecific, Con-conspecific.

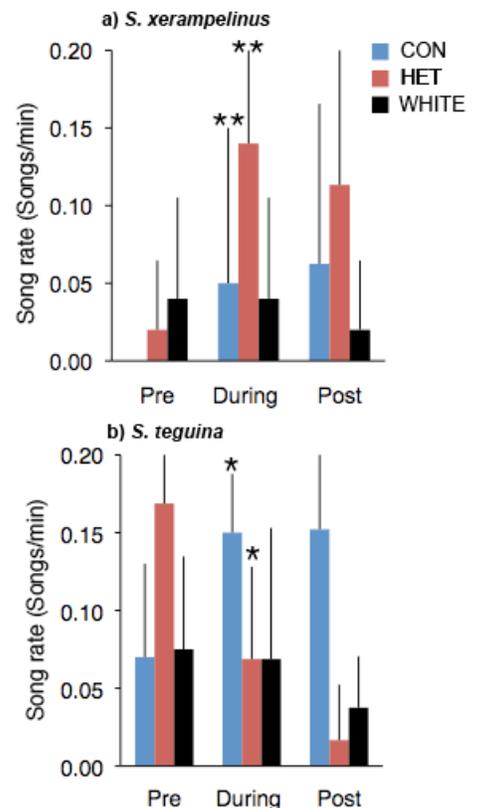


Fig. 7. Response of a) *S. xerampelinus* (n = 8/ trt) and b) *S. teguina* (n = 8/ trt) to playback stimuli in the lab. WHITE-control, HET-heterospecific, CON-conspecific. \*\*  $P > 0.05$ , \*  $P > 0.01$

restricted to colder, more uniform microenvironments, whereas *S. teguina* has the ability to tolerate a wide range of ambient temperatures and conditions. I am submitting a grant to the National Geographic Society to survey *S. teguina* in México and Guatemala. I predict to find *S. teguina* living in colder temperatures at higher altitudes in the absence of *S. xerampelinus*. Results from behavioral interactions in arenas and playback experiments suggest that *S. xerampelinus* is behaviorally dominant to *S. teguina* and song is used in male-male aggression. Below, I describe two aims that focus on behavioral and neural aspects of song perception in sympatry and allopatry.

## Proposed Research

### AIM I. Neural mechanisms of song perception in sympatry

*Rationale*- Classic work in neuroethology describes how the sensory periphery can filter out signals of sympatric heterospecifics (Capranica and Moffat 1983), an appropriate strategy when stimuli are uninformative. However, when closely related species compete, detection and translation of heterospecific signals into distinct motivational responses is crucial. In Aim I, I propose to localize and quantify *Egr-1* (*ZENK*) expression, used extensively in the study of birdsong (Jarvis and Nottebohm 1997, McKenzie et al. 2006), to identify neurons important in song perception. The general design of my study will be to compare regional *Egr-1* induction in brains of sympatric male *S. teguina* and *S. xerampelinus* following playbacks of conspecific (CON), heterospecific (HET), and control (WHITE) stimuli.

*Hypothesis I*- Neural responses to song reflect interspecific dominance in sympatry.

*Predictions*- Playback of *S. teguina* songs to *S. xerampelinus* will induce *Egr-1* expression in the lateral septum (LS), anterior hypothalamus (AH), and medial amygdala (MA), brain regions implicated in aggression (Nelson and Trainor 2007). Playback of *S. xerampelinus* songs to *S. teguina* will induce IEG expression in the central amygdala (CA), a brain region implicated in regulation of fear (Davis 1992). I also predict that playback of *S. teguina* songs to conspecifics and *S. xerampelinus* songs to conspecifics will induce IEG expression in the LS, AH, and MA.

*Methods & Analysis*- Fixed brains from Preliminary Study E will be cryoprotected by transfer into 4% paraformaldehyde + 30% sucrose for 48h. Brains will be stored in a  $-80^{\circ}\text{C}$  freezer until processing for immunocytochemistry. I will collect five sets of 40  $\mu\text{m}$  sections from the forebrain and midbrain at 200 $\mu\text{m}$  intervals. Floating sections will be washed in 3% H<sub>2</sub>O<sub>2</sub> in PBS, blocked in 10% normal goat serum with 3% Triton-X in PBS, and incubated overnight at 4 $^{\circ}\text{C}$  with a goat polyclonal antibody directed against *Egr-1* (Santa Cruz Biotech, 1:300). Control sections will be incubated with a primary antibody inactivated by preincubation with the peptide used in antibody production. Next, sections will be incubated for 1 hr with biotinylated rabbit anti-goat IgG (Leinco), followed by 1 hr incubation in avidin-biotin horseradish peroxidase solution, both diluted in blocking solution. Sections will be immersed in DAB/nickel chloride/0.3% H<sub>2</sub>O<sub>2</sub> and developed for 2-6 min.

I will quantify *Egr-1* expression in four regions of the “social behavior network” (LA, AH, MA, and CA; Goodson 2005) using a morphometric microscope. Each region of interest will be outlined at low power (5-10 x) for the random placement of counting boxes. Cell counts at high power (40-60 x) will be optimized to a minimum of 150 cells/region of interest. I will also count the total number of cells to control for potential between-region differences in cell density. I will use an ANOVA to test for treatment effects in a given brain region, and pair-wise *t*-tests to compare responses to different stimuli. Alpha levels will be corrected for multiple comparisons (Benjamini and Hochberg 1995).

**Challenges & Alternatives-** One possible challenge stems from the fact that neuronal activity is not always followed by *Egr-1* expression (Clayton 2000). Although our *Egr-1* preliminary data is promising (Fig. 8), we can use *c-Fos* as an alternative marker.

## AIM II. Behavioral response and neural mechanisms of song perception in allopatry.

**Rationale-** In a number of species, receivers discriminate less acutely between conspecific and heterospecific stimuli in allopatry (e.g. Jang and Gerhardt 2006). However, few studies have explored the motivational mechanisms of such displacement in social cognition. In Aim II, I propose to study behavioral and neural mechanisms of song perception in an allopatric population of *S. teguina*. I will playback CON, HET, and WHITE stimuli in the field and quantify *Egr-1* expression in response to playbacks in the lab.

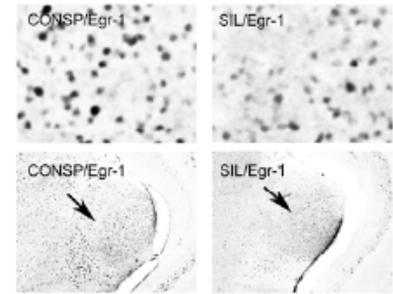


Fig. 8 *Egr-1* expression in the medial geniculate (auditory pathway). Left panels are from *S. teguina* played conspecific songs (LEFT panels) and silence (RIGHT panels).

**Hypothesis IIA-** Sympatric responses to song result from interspecific dominance interactions.

**Prediction-** Allopatric *S. teguina* will respond to songs of both conspecifics and heterospecifics.

**Methods & Analysis-** I will conduct field playback experiments at Cerro La Carpintera (Fig. 1), the closest (18km) allopatric site of *S. teguina* from Volcán Irazú. I will set transects of Sherman live-traps at 10m intervals throughout the study area. Traps will be baited with peanut butter and oats and set open from 7am to 4pm. I will identify locations with a single resident male based on recapture data. I define 'resident' as any animal captured at least four times at a given trap location. Based on capture data, I will choose 12 sites separated by >100m to conduct field playback experiments. My playback protocol will follow methods described in Preliminary Study D, with the exception of calibrating the amplitude of playbacks to match averages for *S. teguina* songs at La Carpintera. CON playback stimuli were originally recorded from males at La Carpintera in winter 2007. HET stimuli will be chosen randomly from our Volcán Irazú song library. All devices used to broadcast songs have a dynamic range (> 40 kHz) that captures the full spectrum of fundamental frequencies for both species. For a given stimulus, responses will be compared between allopatric and sympatric sites. As in Preliminary Study D, I expect *S. teguina* to respond to CON stimuli significantly more than to the WHITE stimuli. This will be reflected in shorter latencies to sing and more songs evoked in response to CON. Allopatric *S. teguina* should respond more often to heterospecific songs than sympatric mice in Aim I. Response latencies and numbers of songs will be analyzed using a GLMs, with ecology as an additional independent variable (sympatry/allopatry).

**Challenges & Alternatives-** A minor challenge has been conducting playback experiments with electrical equipment in a wet cloud forest. I plan to arrive prior to the onset of the wet season next year to reduce the probability of equipment failure. I may find that *S. teguina* does not respond to heterospecific calls in allopatry, which would refute *HIIa*. This could occur if *S. teguina* is easily able to distinguish HET songs from CON songs. Nevertheless, we still ask how the nervous system makes such distinctions in *HIIb* as patterns of neuronal activation could clarify such responses.

**Hypothesis IIB-** Neural responses to song reflect emancipation from interspecific dominance in sympatry.

**Predictions-** Playback of both CON (*S. teguina*) and HET (*S. xerampelinus*) songs to *S. teguina* will induce *Egr-1* expression in aggression circuits (LS, AH, and MA).

**Methods & Analysis-** I will trap 24 separate resident males following the same procedure listed above. Animals will be assigned randomly to 1 of 3 playback treatments: CON, HET, or WHITE (white noise matched to the amplitude and duration of CON). Animals will be isolated in soundproof chambers for 24 hrs after capture, and playbacks will follow the protocol described in Preliminary Study E, with the exception of calibrating the amplitude of playbacks to match averages for *S. teguina* songs at La Carpintera. As in IIA, CON playback stimuli were originally recorded from males at La Carpintera in winter 2007 and HET stimuli will be chosen randomly from our Volcán Irazú song library. I expect *S. teguina* to respond to CON and HET stimuli significantly more than to the WHITE stimuli. This will be reflected in more songs evoked in response to CON. Fixed brains will be prepared and analyzed following methods listed in Aim I. I will use repeated-measures ANOVA to analyze pre-, during, and post responses to each stimulus. We will use Spearman's rank coefficients to compare *Egr-1* staining with number of songs elicited during the trials.

**Challenges & Alternatives-** As mentioned previously, neuronal activity is not always followed by *Egr-1* expression. Although prospects of *Egr-1* are encouraging, we can use *c-Fos* as an alternative marker.

### Summary

Below I briefly summarize predictions related to neural aspects of Aims I and II.

Structure		Sympatry						Allopatry		
Aggression		<i>S. xerampelinus</i>			<i>S. teguina</i>			<i>S. teguina</i>		
		CON	HET	WHITE	CON	HET	WHITE	CON	HET	WHITE
	LS	●	●		●			●	●	
	AH	●	●		●			●	●	
MA	●	●		●			●	●		
Fear	CA					●				

Table 1. Summary of predicted *Egr-1* responses to playbacks in sympatry and allopatry.

### INTELLECTUAL MERIT

The integrative nature of the study enables us to link changes in perception at the neural level to changes in distribution at the geographic level. Examining the mechanisms and function of mammalian song presents exciting opportunities to identify convergent and divergent pathways of perception in birds and other vertebrate taxa. From an applied perspective, insight into the role of interspecific competition in limiting species distributions has direct application to atmospheric warming scenarios. Rising temperatures are predicted to force montane species upward to eventual extinction. Resistance to this 'escalator effect' will depend in part on a species competitive ability (Marris 2007). Thus, incorporating species interactions into climate models will improve our ability to identify taxa most vulnerable to global warming.

**BROADER IMPACTS**

*International Networks & Outreach*

I am committed to providing research opportunities to underrepresented groups in science. During my master’s research, I recruited and provided training to 5 undergraduate students (including 2 U.S. women and 1 Columbian male), all of whom went on to enroll in graduate programs in biology. I recently sponsored a Panamanian colleague’s membership to the American Society of Mammalogists and continue to seek opportunities to collaborate with Latin American biologists. Last summer, I recruited a Costa Rican undergraduate and a local farmer to assist in data collection and presentation. I also conducted public education and outreach in the local communities where I work. Here, I emphasized hypothesis-driven inquiry into the function of animal behavior and underscored the importance of cloud forest conservation. My ultimate goal is to inspire pride and a sense of stewardship for land and animals.



Enjoying a singing mouse call at Escuela División, Cerro de la Muerte

*Local Involvement & Education*

As a member of the University of Florida community, I am similarly committed to the involvement and education of underrepresented groups. Last year, I mentored a female undergraduate who is now applying to graduate school. I am currently mentoring an African-American undergraduate who was recently granted a McNair Fellowship from the US Department of Education. The McNair scholars program prepares underrepresented undergraduate students in science for pursuit of a doctoral degree by providing money, mentoring, and research experience. He and I are conducting an androgen implant study to understand the hormonal control of song in singing mice. I am also working with my committee member, Dr. Doug Levey, to develop an interactive module on animal behavior in his NSF-funded SPICE (Science Partners in Inquiry-based Collaborative Education) program. I will bring in mice from our lab colony at the University of Florida to middle school classrooms with high minority enrollments and incorporate audio and video from my playback experiments to discuss alternative hypothesis-testing and links between the ecosystem, organism, and brain.



Students and mice at Escuela La Pastura, Volcán Irazú

**TIMELINE**

The proposed research can be completed in 1 year. I will have lab assistance from two undergraduates that I am currently training to slice brains and run immunocytochemistry. Below is a timeline for the research aims and educational outreach outlined above.

Spring 2009	Summer 2009	Fall 2009	Spring 2010
Aim I	Aim II	Aim II	Aims I & II
Cut brains, perform immunocytochemistry	Field & lab playback experiments in Costa Rica	Cut brains, perform immunocytochemistry	Finish data analysis, write manuscripts
SPICE module	Visit schools in Costa Rica	SPICE module	

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**STEVEN M. PHELPS**  
**Assistant Professor, Fall 2002-present**  
Department of Zoology  
University of Florida  
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**SUMMER 2008**

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**EDUCATION:**

NSF Center for Behavioral Neuroscience, Emory University 2000-2002  
Position: Postdoctoral fellow  
Topic: Neurobiological and genetic mechanisms of monogamy.  
Sponsors: Drs. Larry J. Young and Thomas R. Insel.

Smithsonian Tropical Research Institute 1999-2000  
Position: Postdoctoral fellow  
Topic: Cognitive models of mate choice in túngara frogs.  
Sponsor: Dr. A. Stanley Rand.

U. Texas, Austin, Ph.D. in zoology/integrative biology 1994-1999  
Position: Postdoctoral fellow  
Dissertation: Historical influences on receiver biases -- neural network simulations and behavioral studies of call recognition in the túngara frog.  
Supervisors: Drs. Michael J. Ryan and Walter Wilczynski.

U. Illinois, Urbana-Champaign, M.S. in biology, 1993-1994  
Thesis: *In vivo* and *in vitro* studies of a putative neurotrophic factor, Dopamine-Releasing Protein.  
Supervisor: Dr. Victor D. Ramirez.

U. Illinois, Urbana-Champaign, B.S. in physiology 1988-1992

**GRANTS:**

P.I. National Science Foundation, \$745,000 *this proposal*  
"CAREER: Integrative neurobiology of species recognition"

P.I. National Science Foundation, \$430,000 *pending*  
"The integrative biology of mating systems"

P.I. National Institutes of Health, \$365,000 *pending*  
"An animal model of parental care: Manipulating social environment with V1aR-RNAi"

P.I., National Geographic, \$20,000 2007-2008  
"Historical biogeography of the singing mice"

P.I., National Science Foundation, \$200,000 2006-2008  
"Integrative biology of vocal communication"

P.I., National Science Foundation, \$16,000 2005  
"Workshop on rodents as models for integrative study of social behavior"

P.I., National Science Foundation, \$280,000 2003-2006  
"Behavioral and neuroendocrine variation in mammalian mating systems."

**HONORS AND AWARDS:**

**UF College of Liberal Arts and Sciences Teacher of the Year Award** 2006-2007  
University of Florida Academy of Distinguished Teacher-Scholars, nominee 2006-2007

**NSF Center for Behavioral Neuroscience Postdoc. Fellow** 2000-2002

**Smithsonian Postdoctoral Fellow** 1999

University of Texas Continuing Fellow nominee, declined 1999

University of Texas Tuition Fellowship	1998, 1999
Dorothea Bennet Travel Fellowship, U.T. Dept. Zoology	1996, 1998
<b>Smithsonian Predoctoral Fellow</b>	1998
Dorothea Bennet Research Fellowship, U.T. Dept. Zoology	1995, 1998
<b>National Research Service Award</b> , National Institutes of Health	1995-1998
Endowment for Excellence Fellowship, U.T. Dept. Zoology	1997
Bruton Award, U.T. Graduate School	1996
Sigma Xi Grants-in-Aid	1996
Gaige Award, American Society of Ichthyologists and Herpetologists	1996
National Science Foundation Undergrad. Research Fellowship	1992

### TEN RECENT AND REPRESENTATIVE PUBLICATIONS:

- A.G. Ophir, P. Campbell, K. Hannen and **S.M. Phelps**. *In press*. Field tests of *cis*-regulatory variation at the prairie vole *avpr1a* locus: association with V1aR abundance but not social or sexual fidelity. *Hormones and Behavior*.
- P. Campbell, R. Reep, M. Stoll, A. Ophir and **S.M. Phelps**. *In press*. Conservation and diversity of *Foxp2* expression in muroid rodents: Functional implications. *Journal of Comparative Neurology*.
- S.M. Phelps** and A.G. Ophir. *In press*. Monogamous brains and alternative tactics: Neuronal V1aR, space use and sexual infidelity among male prairie voles. *Cognitive Ecology*, Reuven Dukas Ed. 2<sup>nd</sup> edition. University of Chicago Press.
- A.G. Ophir, J.O. Wolff and **S.M. Phelps**. 2008. Variation in neural V1aR predicts space use and sexual fidelity among male prairie voles in semi-natural settings. *Proceedings of the National Academy of Sciences* 105:1249-1254.
- A.G. Ophir, A.B. Sorin, **S.M. Phelps** and J.O. Wolff. 2008. Social but not genetic monogamy is associated with higher breeding success in prairie voles. *Animal Behavior* 75:1143-1154.
- S.M. Phelps**. 2006. The integrative biology of social behavior: Rodents as extended model systems. *National Science Foundation Workshop Report*.
- S.M. Phelps**, A.S. Rand and M.J. Ryan. 2006. The cognitive architecture of mate choice and species recognition. *American Naturalist* 167:28-42.
- S.M. Phelps** and L.J. Young. 2003. Extraordinary diversity in vasopressin (V1a) receptor expression in wild prairie voles: Patterns of variation and covariation. *Journal of Comparative Neurology* 466:564-576.
- S.M. Phelps**, M.J. Ryan and A.S. Rand. 2001. Vestigial preferences in neural networks and túngara frogs. *Proceedings of the National Academy of Sciences* 98:13161-13166.
- S.M. Phelps** and M.J. Ryan. 2000. History influences signal recognition: Neural network models of túngara frogs. *Proceedings of the Royal Society B* 267:1633-1639.

### SYNERGISTIC ACTIVITIES – TEACHING, MENTORING AND OUTREACH:

<i>Courses taught</i>	Introductory biology: Integrative principles 800 students	fall 2002-present
	Comparative and evolutionary neurobiology	spring 2007, 2008
	Evolution of brain size	spring 2006
	Communication and signaling: from cells to systems	fall 2003-2005
	Mechanisms of behavior	spring 2005, 2007
	Evolution of gene regulation	spring 2005
	Historical readings in animal behavior	spring 2004
<i>Minority mentorship</i>	University Minority Mentor Program	fall 2005-present
	McNair Scholars Program	fall 2006-present
<i>Outreach</i>	Science Partners in Inquiry-based Collaborative Education	fall 2006-present

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Website: [www.zoology.ufl.edu/bpasch](http://www.zoology.ufl.edu/bpasch)

## EDUCATION

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University of Florida, Gainesville, FL	<b>Ph.D.</b> Zoology	Current
University of Arizona, Tucson, AZ	<b>M.S.</b> Wildlife Ecology	2001-2004
Universidad de Costa Rica OTS-1 Tropical Biology: An Ecological Approach		2004
Ursinus College, Collegeville, PA	<b>B.S.</b> Honors in Biology	1997-2001

## SELECTED GRANTS, HONORS, & AWARDS

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<b>Society for Integrative &amp; Comparative Biology Grants-In-Aid</b>	2008
<b>American Society of Mammalogists Grants-In-Aid</b> <b>Sigma Xi Grants-In-Aid of Research</b> <b>Riewald-Olowo Award</b> , University of Florida Department of Zoology	2007
<b>Alumni Fellowship</b> , College of Liberal Arts & Sciences, UF	2006
<b>Roger Hungerford Award</b> , Arizona Wildlife Society	2005
<b>Post-Course Research Grant</b> , Organization for Tropical Studies	2004
<b>Howard McCarley Research Grant</b> , SW Association of Naturalists <b>Conservation Biology Research Grant</b> , T & E, Inc.	2003
<b>Phi Beta Kappa Society</b>	2001

## PUBLICATIONS

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- Pasch, B.**, and J.L. Koprowski. 2006. Sex differences in space use of Chiricahua fox squirrels. *Journal of Mammalogy* 87: 380-386.
- Pasch, B.**, and J.L. Koprowski. 2006. Annual cycles in body mass and reproduction of Chiricahua fox squirrels. *SW Naturalist* 51: 531-535.
- Koprowski, J.L., Ramos-Lara, N., **Pasch, B.**, and C. Zugmeyer. 2006. Observations on the ecology of the endemic Mearns's Squirrel (*Tamiasciurus mearnsi*). *SW Naturalist* 51: 426-430.
- Pasch, B.**, and J.L. Koprowski. 2005. Correlates of vulnerability in Chiricahua fox

- squirrels. Pp. 426-428 in *Connecting Mountain Islands and Desert Seas: Biodiversity and Management of the Madrean Archipelago II* (G.J. Gottfried, B.S. Gebow, L.G. Eskew, and C.B. Edminster, eds.) Rocky Mountain Research Station, Fort Collins, Colorado.
- Koprowski, J.L., Edelman, A., **Pasch, B.**, and D. Buecher. 2005. A dearth of data on mammals of the Madrean Archipelago: What we think we know and what we actually do know. Pp. 412-415 in *Connecting Mountain Islands and Desert Seas: Biodiversity and Management of the Madrean Archipelago II* (G.J. Gottfried, B.S. Gebow, L.G. Eskew, and C.B. Edminster, eds.) Rocky Mountain Research Station, Fort Collins, Colorado.
- Pasch, B.**, and J.L. Koprowski. In review. Fire suppression on montane islands: Impacts on an endemic tree squirrel. *Journal of Mammalogy*.
- Leonard, K.M., **Pasch, B.**, and J.L. Koprowski. In review. *Sciurus pucheranii*. *Mammalian Species*.

### **TEACHING, MENTORING, AND OUTREACH**

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#### *Courses led at the University of Florida:*

- |   |             |
|---|-------------|
| Altitudinal gradients in ecology<br>Sponsor: Dr. Doug Levey | Fall 2007   |
| Current topics in mammalogy<br>Sponsor: Dr. Polly Campbell  | Spring 2007 |

#### *Minority mentorship:*

- |                         |                       |
|-------------------------|-----------------------|
| McNair Scholars Program | Summer 2008 - present |
|-------------------------|-----------------------|

#### *Outreach:*

- |   |                       |
|---|-----------------------|
| Hands-on workshops at elementary and middle schools in Costa Rica | Summer 2007 - present |
|---|-----------------------|

# SUMMARY PROPOSAL BUDGET

YEAR 1

ORGANIZATION <b>University of Florida</b>				FOR NSF USE ONLY			
				PROPOSAL NO.	DURATION (months)		
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR <b>Steven M Phelps</b>				AWARD NO.	Proposed	Granted	
A. SENIOR PERSONNEL: PI/PI, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)				NSF Funded Person-months		Funds Requested By proposer	Funds granted by NSF (if different)
				CAL	ACAD	SUMR	
1.	<b>Steven M Phelps - none</b>			0.00	0.00	0.00	\$ 0 \$
2.	<b>Bret S Pasch - none</b>			0.00	0.00	0.00	0
3.							
4.							
5.							
6.	( 0 ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)			0.00	0.00	0.00	0
7.	( 2 ) TOTAL SENIOR PERSONNEL (1 - 6)			0.00	0.00	0.00	0
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)							
1.	( 0 ) POST DOCTORAL SCHOLARS			0.00	0.00	0.00	0
2.	( 0 ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)			0.00	0.00	0.00	0
3.	( 0 ) GRADUATE STUDENTS						0
4.	( 0 ) UNDERGRADUATE STUDENTS						0
5.	( 0 ) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)						0
6.	( 0 ) OTHER						0
TOTAL SALARIES AND WAGES (A + B)							0
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)							0
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)							0
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)							
	<b>ACO Pacific 7016 microphone</b>		\$ 1,500				
	<b>Avisoft RECORDER software (#10302)- educational price</b>		1,000				
	<b>Avisoft UltraSoundGate Player 116 (#70111)- educational price</b>		3,100				
TOTAL EQUIPMENT							5,600
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)							550
2. FOREIGN							2,250
F. PARTICIPANT SUPPORT COSTS							
1.	STIPENDS \$ _____		0				
2.	TRAVEL _____		0				
3.	SUBSISTENCE _____		0				
4.	OTHER _____		0				
TOTAL NUMBER OF PARTICIPANTS ( 0 ) TOTAL PARTICIPANT COSTS							0
G. OTHER DIRECT COSTS							
1.	MATERIALS AND SUPPLIES						3,800
2.	PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION						0
3.	CONSULTANT SERVICES						0
4.	COMPUTER SERVICES						0
5.	SUBAWARDS						0
6.	OTHER						2,800
TOTAL OTHER DIRECT COSTS							6,600
H. TOTAL DIRECT COSTS (A THROUGH G)							15,000
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) (Rate: , Base: )							
TOTAL INDIRECT COSTS (F&A)							0
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)							15,000
K. RESIDUAL FUNDS							0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)							\$ 15,000 \$
M. COST SHARING PROPOSED LEVEL \$ 0				AGREED LEVEL IF DIFFERENT \$			
PI/PI NAME <b>Steven M Phelps</b>				FOR NSF USE ONLY			
ORG. REP. NAME* <b>Brian prindle</b>				INDIRECT COST RATE VERIFICATION			
		Date Checked	Date Of Rate Sheet	Initials - ORG			

# SUMMARY PROPOSAL BUDGET

Cumulative

ORGANIZATION <b>University of Florida</b>				FOR NSF USE ONLY			
				PROPOSAL NO.	DURATION (months)		
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR <b>Steven M Phelps</b>				AWARD NO.	Proposed	Granted	
				A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)			
				CAL	ACAD	SUMR	
1. <b>Steven M Phelps - none</b>				0.00	0.00	0.00	\$ 0
2. <b>Bret S Pasch - none</b>				0.00	0.00	0.00	0
3.							
4.							
5.							
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)				0.00	0.00	0.00	0
7. ( <b>2</b> ) TOTAL SENIOR PERSONNEL (1 - 6)				0.00	0.00	0.00	0
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)							
1. ( <b>0</b> ) POST DOCTORAL SCHOLARS				0.00	0.00	0.00	0
2. ( <b>0</b> ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)				0.00	0.00	0.00	0
3. ( <b>0</b> ) GRADUATE STUDENTS							0
4. ( <b>0</b> ) UNDERGRADUATE STUDENTS							0
5. ( <b>0</b> ) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)							0
6. ( <b>0</b> ) OTHER							0
TOTAL SALARIES AND WAGES (A + B)							0
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)							0
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)							0
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)							
				\$	5,600		
TOTAL EQUIPMENT							5,600
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)							550
2. FOREIGN							2,250
F. PARTICIPANT SUPPORT COSTS							
1. STIPENDS \$ _____				0			
2. TRAVEL _____				0			
3. SUBSISTENCE _____				0			
4. OTHER _____				0			
TOTAL NUMBER OF PARTICIPANTS ( <b>0</b> )							
TOTAL PARTICIPANT COSTS							0
G. OTHER DIRECT COSTS							
1. MATERIALS AND SUPPLIES							3,800
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION							0
3. CONSULTANT SERVICES							0
4. COMPUTER SERVICES							0
5. SUBAWARDS							0
6. OTHER							2,800
TOTAL OTHER DIRECT COSTS							6,600
H. TOTAL DIRECT COSTS (A THROUGH G)							15,000
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)							
TOTAL INDIRECT COSTS (F&A)							0
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)							15,000
K. RESIDUAL FUNDS							0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)							\$ 15,000
M. COST SHARING PROPOSED LEVEL \$ <b>0</b>				AGREED LEVEL IF DIFFERENT \$			
PI/PD NAME <b>Steven M Phelps</b>				FOR NSF USE ONLY			
ORG. REP. NAME* <b>Brian prindle</b>				INDIRECT COST RATE VERIFICATION			
		Date Checked		Date Of Rate Sheet		Initials - ORG	

C \*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

## Budget Justification

The proposed research aims require durable electronic equipment for playback experiments in the field and rustic labs. My preliminary data was collected with equipment that has since failed due to repeated use in the wet cloud forests. Thus, I am requesting funds to purchase more robust equipment that will allow me to complete my Ph.D. work and initiate future bioacoustic studies. Major items include a microphone (ACO Pacific 7016 microphone and PS9200 preamp, \$2000), power amplifier (Avisoft UltraSoundGate Player; \$3100), sound level meter (Extech; \$400), high-frequency speakers (Avisoft Ultrasonic Dynamic Speaker ScanSpeak; \$800), and a digital recorder (Fostex FR2-LE; \$700) designed for use in the field.

The PI and colleagues in the Veterinary School at the University of Florida provide space, most bench supplies, and use of a microtome and morphometric microscope for neuroanatomical analysis. I am requesting funds to purchase materials to fix brains while in the field and specific antibodies (*egr-1*; \$400 Santa Cruz Biotechnologies) to stain brains in the lab.

Although I have acquired a cursory bioacoustics skill set from trial and error in the lab and field, I would benefit from more formal training on permutations of playback design, spectrogram analysis, and computer coding. Thus, I request funding to attend a Sound Analysis Workshop presented by the Bioacoustics Research Program at Cornell University to increase my proficiency in bioacoustics (airfare and registration; \$2050).

Finally, a major goal of my research is to educate members of the local communities where I work and forge collaborations with Latin American biologists. Hence, I am requesting funds for transportation to my field site (airfare and bus; \$750) to collect data and revisit two Costa Rican elementary schools where I will continue outreach. I am also requesting funds to disseminate my research in the "Acoustic communication in terrestrial and aquatic mammals" symposium at the 10th International Mammalogical Conference in Mendoza, Argentina (airfare; \$1500). The UF Department of Zoology will provide \$300 to assist in registration and travel.

## Current and Pending Support

(See GPG Section II.C.2.h for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.	
Investigator: Steven Phelps	Other agencies (including NSF) to which this proposal has been/will be submitted.
Support: <input type="checkbox"/> Current <input checked="" type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support Project/Proposal Title: Collaborative Research: An integrative approach to the neuroendocrine basis for mammalian mating systems  Source of Support: National Science Foundation Total Award Amount: \$ 430,000 Total Award Period Covered: 09/01/08 - 08/31/12 Location of Project: University of Florida Person-Months Per Year Committed to the Project.   Cal:0.00   Acad: 0.00   Sumr: 1.00	
Support: <input type="checkbox"/> Current <input checked="" type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support Project/Proposal Title: An animal model of parental care: Manipulating social environment with V1aR-RNAi.  Source of Support: National Institutes of Health Total Award Amount: \$ 365,000 Total Award Period Covered: 01/01/09 - 12/31/10 Location of Project: University of Florida Person-Months Per Year Committed to the Project.   Cal:0.00   Acad: 0.00   Sumr: 1.00	
Support: <input type="checkbox"/> Current <input checked="" type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support Project/Proposal Title: CAREER: The integrative neurobiology of species recognition.  Source of Support: National Science Foundation Total Award Amount: \$ 744,777 Total Award Period Covered: 01/01/09 - 12/31/14 Location of Project: University of Florida Person-Months Per Year Committed to the Project.   Cal:0.00   Acad: 0.00   Sumr: 1.00	
Support: <input type="checkbox"/> Current <input type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support Project/Proposal Title:  Source of Support: Total Award Amount: \$                      Total Award Period Covered: Location of Project: Person-Months Per Year Committed to the Project.   Cal:              Acad:              Sumr:	
Support: <input type="checkbox"/> Current <input type="checkbox"/> Pending <input type="checkbox"/> Submission Planned in Near Future <input type="checkbox"/> *Transfer of Support Project/Proposal Title:  Source of Support: Total Award Amount: \$                      Total Award Period Covered: Location of Project: Person-Months Per Year Committed to the Project.   Cal:              Acad:              Summ:	

\*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.



## FACILITIES, EQUIPMENT & OTHER RESOURCES

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**FACILITIES:** Identify the facilities to be used at each performance site listed and, as appropriate, indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Use "Other" to describe the facilities at any other performance sites listed and at sites for field studies. USE additional pages as necessary.

**Laboratory:** -80 freezer  
2 small -20 chest freezers  
1 upright refrigerator/freezer  
Eppendorf pipettors and 24 well cell culture plates for

**Clinical:**

**Animal:** Two animal housing rooms, one with a colony of singing mice (*S. teguina*, *S. xerampelinus*), the other with a colony of prairie voles. Colonies are monitored by veterinary staff. 20 collapsible PVC-coated wire cages, bottoms, food and water bowls for field housing.

**Computer:** Hardware:  
3 Dell desktop computers  
2 Dell laptop computer  
Scanner, resolution 2400dpi

**Office:** Desk space for 2 postdocs, department supplies, and office space for 4 graduate students.

**Other:**

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**MAJOR EQUIPMENT:** List the most important items available for this project and, as appropriate identifying the location and pertinent capabilities of each.

The following items are within our laboratory:  
Leica DMIL research grade microscope  
Leica 1900 cryostat

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**OTHER RESOURCES:** Provide any information describing the other resources available for the project. Identify support services such as consultant, secretarial, machine shop, and electronics shop, and the extent to which they will be available for the project. Include an explanation of any consortium/contractual arrangements with other organizations.

Software:  
Adobe Photoshop, Illustrator, Acrobat, Premiere  
Microsoft Office  
MatLab (includes software written for behavioral video analysis, sound analysis)  
J-Watcher (video scoring software)  
Image-J

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## FACILITIES, EQUIPMENT & OTHER RESOURCES

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Continuation Page:

LABORATORY FACILITIES (continued):

immunohistochemistry

COMPUTER FACILITIES (continued):



College of Liberal Arts & Sciences  
Department of Zoology

Room 223 Bartram Hall  
PO Box 118525  
Gainesville, FL 32611-8525  
352-392-1107  
352-392-3704 Fax

11 November 2008

TO: NSF DDIG Review Committee

RE: Bret Pasch Candidacy Status

THIS STUDENT HAS ADVANCED TO CANDIDACY FOR A Ph.D. DEGREE.

Sincerely,

A handwritten signature in blue ink that reads 'Karen A. Bjorndal'. The signature is written in a cursive style and is positioned above a faint, light-colored rectangular stamp.

Karen A. Bjorndal  
Professor and Chair

## **Context for Improvement**

The proposed study forms an integral part of my dissertation research. The ecological and behavioral data that I collected over the past two years provides an important foundation for the mechanistic questions proposed in this grant. Although I have already conducted playback experiments and collected brains at sympatric sites, I currently lack funding to analyze immediate early gene expression. More importantly, I will need NSF funding to repeat my experiments in allopatry to sufficiently address the evolution of neural mechanisms of perception. NSF funding would substantially (and literally) improve the breadth (allopatry vs. sympatry) and depth (behavioral response vs. neural underpinnings) of my dissertation. The proposed research will also provide me with the tools, insights, and collaborations that will form the basis of my future long-term research program.

My proposed research is independent of Steve Phelps' (PI) past and current work on singing mice. While his past work focused on how song functions in mate attraction, my studies explicitly address the role of song in male-male interactions in an ecological context. My proposed research is related to his general interest in species recognition and mechanisms of behavior. However, there is currently no overlap in our funding.