

Comparing Within Vs. Between Individual Mating Call Variation in Upland Chorus Frog (*Pseudacris feriarum*) Population

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Abstract

A primary goal of evolutionary biology is to understand the forces driving the generation of Earth's biodiversity through speciation. **Reinforcement**—the evolution of behavioral reproductive isolation between species due to selection against hybridization—is one evolutionary force that can drive speciation by reducing the likelihood of interbreeding between incipient species. Reinforcement can also indirectly cause mating behaviors to **diverge** within a species, which can lead to **reproductive isolation** among populations of the same species. **Chorus frogs** (genus *Pseudacris*) are one group that shows evidence of speciation via reinforcement of male acoustic signals (calls) and female mating preferences in areas of overlap between species. One species, the **Upland chorus frog** (*P. feriarum*), also exhibits call divergence between its populations that do vs. do not overlap with another species, the **Southern chorus frog** (*P. nigrita*). What is unknown is whether *P. feriarum* with these extreme phenotypes recognize each other as the same species or whether they have become behaviorally isolated, thus representing incipient new species? Here, we investigated this question in a population of *P. feriarum* near Macon, Georgia, which contains individuals spanning a broad range of call phenotypes, including extremes. Specifically, we asked whether the call characters **pulse rate (PR)** and **pulse number (PN)** are more variable among different frogs than among calls of a single individual. **We predicted that if individuals with very different call phenotypes from each other have evolved reproductive isolation, their call characters will be more variable among than within individuals.** If, however, extreme individuals are not reproductively isolated, call variation among individuals is expected to be similar to the variation within individuals. To test this idea, we extracted and analyzed calls of 400 frogs from the Macon site, including twenty calls per frog: ten each from the beginning and end of a call bout across two recording sessions. Understanding the extent of individual variation in these call parameters will offer insight into how reproductive behaviors evolve and diversify during speciation by reinforcement.

Background

- Species form when populations evolve reproductive barriers, and reinforcement strengthens these barriers by selection against hybridization (Fouquette 1975)
- Upland and Southern chorus frogs are a good study system for reinforcement because few other taxa are present during their breeding season and they are undergoing secondary contact after having diverged during the Miocene era (Lemmon 2009).
- However, we do not know whether Upland chorus frogs (*P. feriarum*) with these phenotypic differences recognize each other as the same species or whether they have become behaviorally isolated to create new species?
- The project specifically examines how *P. feriarum* with extreme phenotypes recognize other *P. feriarum* as the same species (became behaviorally isolated), or whether reinforcement caused speciation.
- We focused on *P. feriarum* in **Macon, Georgia** (Figure 3) with individuals spanning broad range of call phenotypes. In order to get the most revealing results, we honed in on two call characters: **pulse rate (PR)** and **pulse number (PN)**.
- We know that in overlap zones between species call phenotypic characteristics diverge to prevent hybridization, therefore we predict that within the Macon transect call variance will be higher across different individuals than across calls within each individual frog.**

Methods

- We built soundproof boxes using a power drill, nails, wood, cloth, and “spun rock.”
- We captured 400 male chorus frogs from the panmictic site (Figure 3), put them on ice, and recorded their calls using soundproof boxes in the laboratory.
- We extracted and analyzed ten calls per frog (five from the beginning and five from the end of the call) for two recording sessions using the **ProTools software**.
- After extracting the calls, we ran each call through a call analysis software called **Sound Ruler** to create **Excel** files to conduct further statistical analysis (t-tests) on the variation between calls.
- Using Excel we calculated the variance of both pulse number and pulse rate within an individual frog's calls and between individual frog's calls
- We averaged the variances between pulse rate and number from within individuals and between individuals
- We divided the averages for within individual's calls by the average between individual calls to determine the relative magnitude of variation between one frog's calls versus between multiple frog calls.

Results

- For pulse rate, variation was **4.85 times** higher among frogs than among calls within each frog.
- For pulse number, variation was **3.09 times** higher among frogs than among calls within each frog.
- T-test concluded **PR (p=0.00028; Figure 1)** and **PN (p=0.00059; Figure 2)**

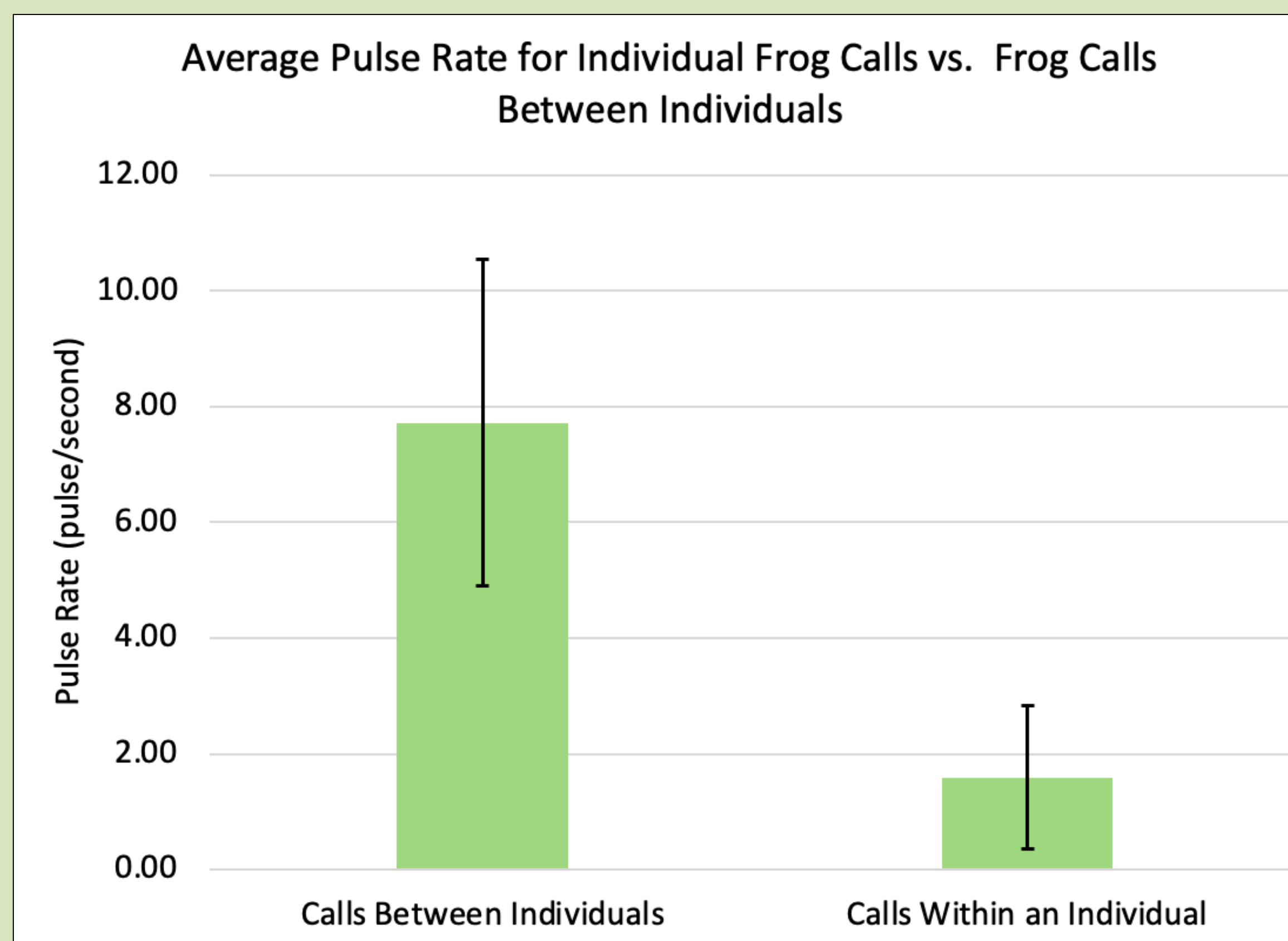


Figure 1: Average pulse rate of *Pseudacris feriarum* from calls between individuals (A) and calls within an individual (B). The error bars represent the 95% confidence intervals of both conditions (A: CI=2.82; B: CI=1.23). Calls between individuals exhibited significantly higher variance than calls within individuals (p=0.000284).

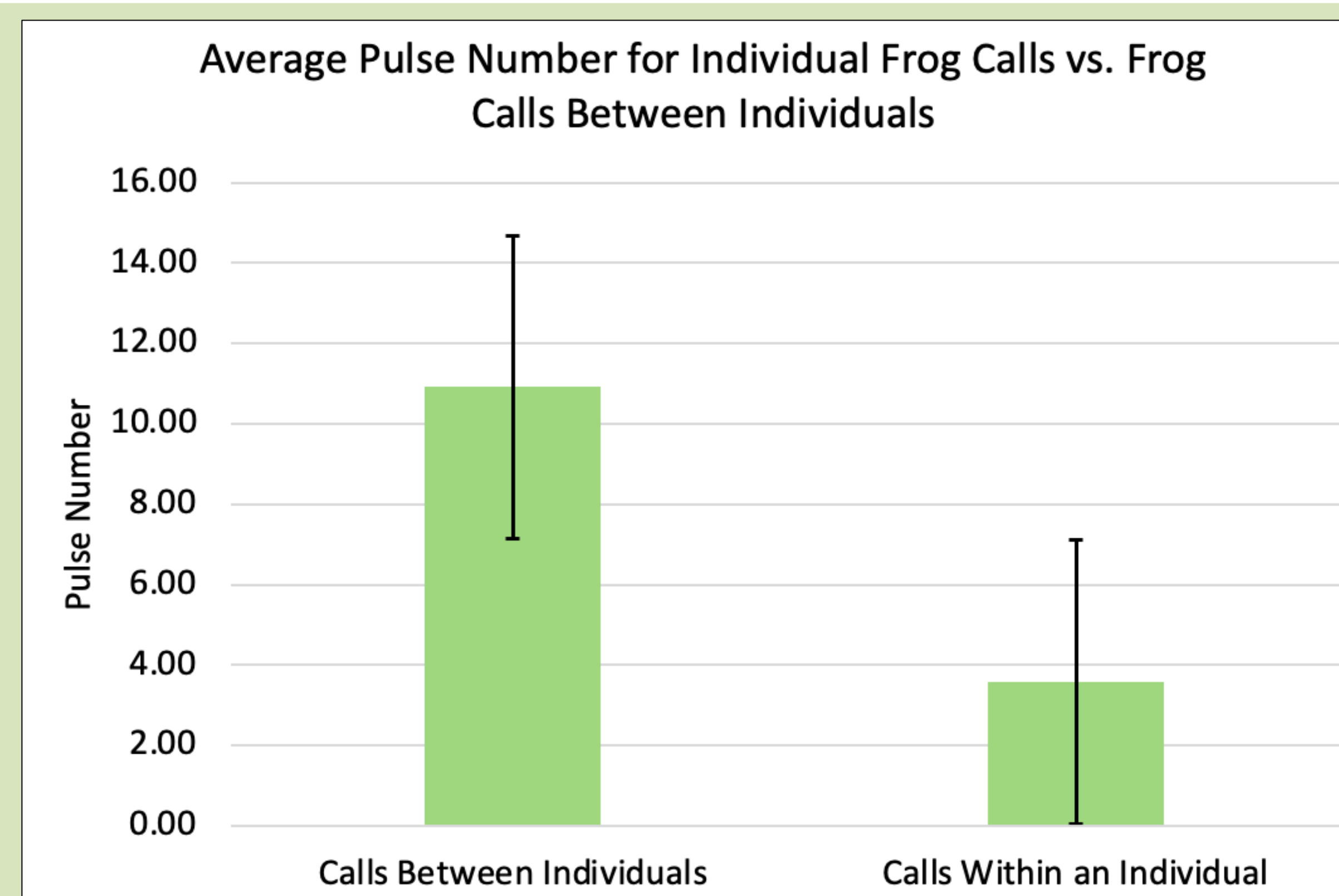


Figure 2: Average pulse number of *Pseudacris feriarum* from calls between individuals (A) and calls within an individual (B). The error bars represent the 95% confidence intervals of both conditions (A: CI=2.79; B: CI=2.62). Calls within an individual exhibited significantly more variance than calls between individuals (p=0.0005955).

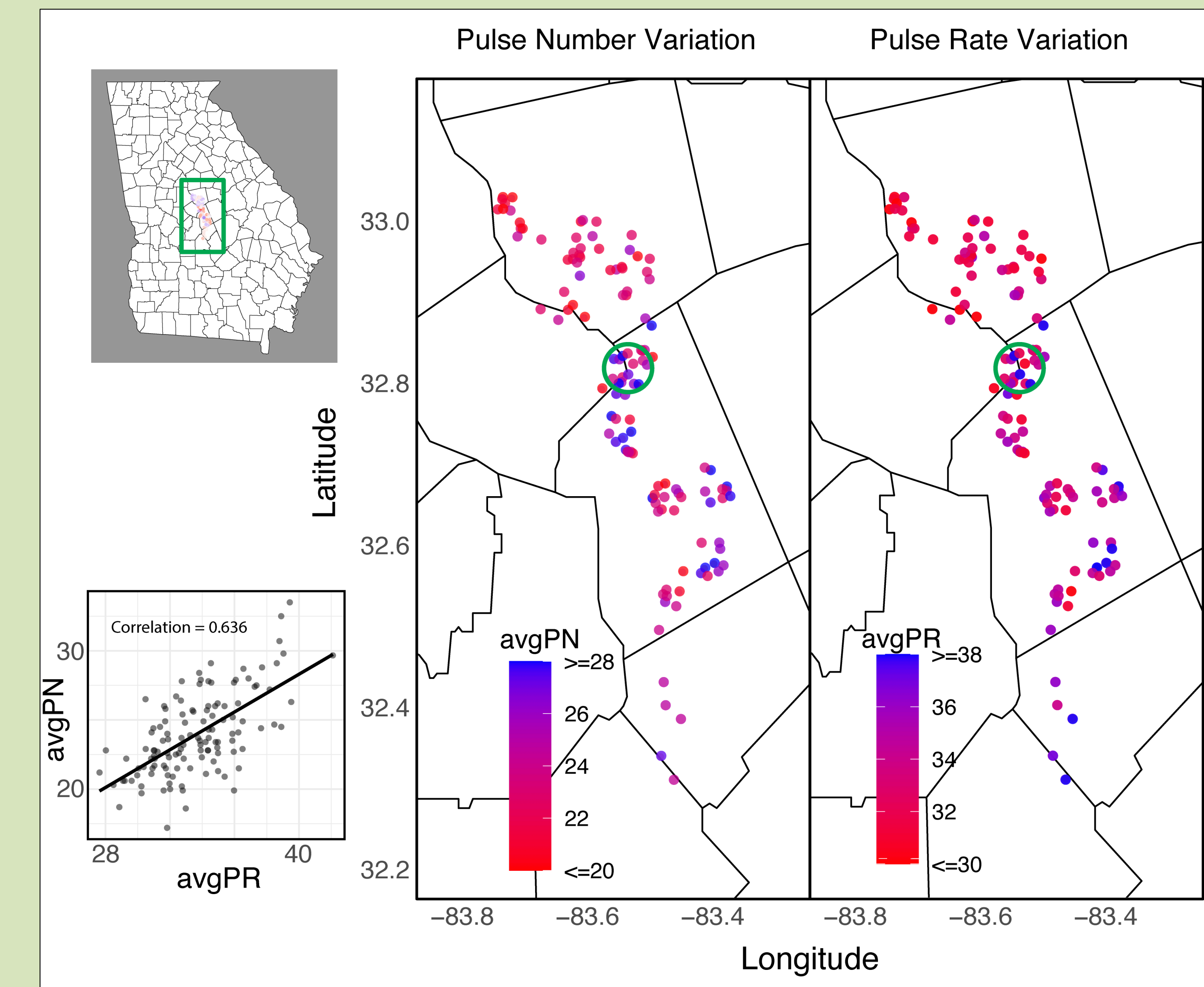


Figure 3: An overview map of Georgia (A), a map of the Macon transect that shows pulse number variation (B), and another map of the Macon transect showing pulse rate variation (C). The green circle shows the sample area in the Macon transect.

Discussion

- Our prediction was upheld by the data in this study: male calls are more variable among than within individuals at the target field site.**
- Our study found that variation among different chorus frogs' calls was significantly larger than the variation within an individual's calls, with pulse rate being **4.85 times** more variable and pulse number **3.09 times** more variable.
- Our results raise the question of whether *P. feriarum* individuals with extreme call phenotypes recognize each other as the same species or if they have become behaviorally isolated, potentially representing an early stage of speciation.
- This suggests mating calls in this population are under selective pressures that drive male frog call divergence in *P. feriarum*, potentially due to sympatric interactions with other chorus frog species such as *P. nigrita*.
- These findings align with existing research on call differentiation as a mechanism for species reinforcement.
- In the future, we aim to conduct a **Genomic-Wide Association Study (GWAS)** to explore the genetic basis of this variation. We aim to gather exact phenotypic data to develop a phenotype-genotype map.
- As we utilized **AI platforms** (specifically **Grok3** for R-based data analysis) the study provides basis for future research to see how machine learning can improve bioacoustic research in the future.



Citations

