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Age-related changes and vocal convergence in titi monkey duet pulses

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Abstract
Duets in pair-bonding primates serve as a primary mode of communication between pairs, and duets may provide cues to conspecifics regarding the calling individual or pair. Here, we test the hypothesis that pulse elements in coppery titi monkey duets vary with condition and identity of the caller. We predicted that pulse elements would vary with age, sex, or pair-bond length. We estimated pulse rate and duration for 378 pulse elements from the duets of 74 captive titi monkeys (*Plecturocebus cupreus*). We found inter-individual variation in both features, and evidence for vocal convergence among pair mates in pulse rate. Age was the best predictor of pulse rate, and pulse rate decreased with age. Age and pair-bond length reliably predicted pulse duration. Our results suggest that variation in titi monkey duets reflects differences in caller condition and pair identity, and contribute to growing evidence for vocal plasticity in nonhuman primates.

Keywords
vocal plasticity, acoustic communication, vocal variation, neotropical primates.

1. Introduction

Acoustic signals mediate social interactions in vocal animals, and play an important role in mate choice, species recognition and resource defence (Wilkins et al., 2012). Acoustic signals can also provide important infor-

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mation to the receiver regarding the signaller's condition, genetic quality, or motivation (Bradbury & Vehrencamp, 1998). Intra-specific variation in acoustic signals can arise through a variety of mechanisms, including genetics, environment and social factors (Wilkins et al., 2012); these mechanisms are not mutually exclusive. Given the strong evidence for genetic influences on primate call morphology (Geissmann, 1984), the traditional view has been that nonhuman primate vocalizations are innate and inflexible, and that nonhuman primates lack the ability for vocal production learning and vocal plasticity (Janik & Slater, 1997), but recent advances have challenged these ideas (reviewed in Snowdon, 2017; Bergman et al., 2019). Although species-specific call structure appears to be influenced by genetic factors, the growing evidence for plasticity in fine scale structure of certain call types suggests that social or other factors also play a role in shaping call structure (Lemasson et al., 2011).

Duets are the stereotyped, coordinated vocalizations between two or more individuals (Dahlin & Benedict, 2014). Various hypotheses for the function of duets in nonhuman primates have been proposed including regulation of intra- and inter-group spacing (Robinson, 1981), joint territory defence, mate-guarding, and strengthening or advertisement of the pair-bond (Marshall-Ball et al., 2006). Coordinated duets may advertise the willingness of individuals to defend shared resources, and provide a demonstration of the resource holding potential of the duetting pair (Robinson, 1981; Caselli et al., 2015). In addition, territorial vocalizations — including individual contributions to duets — may be honest indicators of an individual's resource holding potential, if call features are influenced or constrained by caller morphology and physiology (Vehrencamp, 2000; Smith & Harper, 2003; Terleph et al., 2016).

There is growing evidence for vocal plasticity in the duets of nonhuman primates. For example, male lar gibbons (*Hylobates lar*) are able to flexibly time their duet contributions relative to the female call, and although female calls were of variable length, males replied in close synchrony with the end of the female call (Terleph et al., 2018). Dominant and nondominant indri (*Indri indri*) individuals avoid overlapping during calling (Gamba et al., 2016), providing evidence for vocal flexibility in even the most basal primates. Acoustic convergence at the group-level has been documented in mouse lemurs (*Microcebus murinus*; Hafen et al., 1998), Japanese macaques (*Macaca fuscata*; Tanaka et al., 2006) and chimpanzees (*Pan troglodytes*;

Marshall et al., 1999). In spectral tarsiers (*Tarsius spectrumgurskyae*), duetting partners have the ability to simultaneously modify the rate of note output relative to their partners (Clink et al., 2019), and pygmy marmosets (*Cebuella pygmaea*) modify the structure of their trills when paired, exhibiting vocal convergence among pair mates in trill structure (Snowdon & Elowson, 1999). In certain duetting primates, duet convergence may function in the formation or solidifying of the pair bond, and variation in the vocal structure of duets may encode information about both the pair and individuals (Snowdon, 2009).

Variation in vocal structure may also vary with individual condition, or motivation of the caller. Across diverse taxa, calling rates can be linked to individual metabolic rate, such that call features (including calling rate) vary predictably with body size and temperature (Gillooly & Ophir, 2010; Ophir et al., 2010). But, differences in metabolism do not explain all the variation observed in rate of element repetition, particularly with regard to intra-species comparisons. For example, roaring rates of Scottish red deer stags (*Cervus elaphus*) are highly correlated with the male's fighting ability, and provide an accurate signal of the male's ability to repel intruders (Clutton-Brock & Albon, 1979). In lar gibbons, older females had a lower ratio of note output relative to the breaks between notes, taking longer breaks between notes than younger females (Terleph et al., 2016). In singing mice (*Scotinomys* spp.) androgen levels modulate trill rate, and males with experimentally lowered androgen levels exhibited a decrease in rate of note repetition (Pasch et al., 2011). In male chimpanzees (*Pan troglodytes*), hourly rates of pant-hooting were correlated with testosterone levels (Fedurek et al., 2016).

Rate of element repetition may also be influenced by the motivation of the signaller, as shown via playback studies. In banded wrens (*Thryophilus pleurostictus*), rate of note repetition increased in response to simulated territorial intrusions, showing that animals can flexibly modify their rate of note output according to motivation (Vehrencamp et al., 2013). In wood warblers (*Phylloscopus sibilatrix*), males respond differentially to simulated intrusions depending on intruder song rate, and males increased their song rate during territorial contests (Szymkowiak & Kuczyński, 2017). Java sparrows (*Lonchura oryzivora*) produce "trill-calls" in both aggressive and affiliative contexts, but in aggressive contexts sound pressure levels, rate of note repetition, and entropy are higher (Furutani et al., 2018), providing further evidence that animals modify rate of element repetition in response to motivational context.

Titi monkeys are territorial, socially monogamous neotropical primates that produce elaborate species-specific duets wherein males and females overlap substantially both temporally and structurally in their duet contributions (Robinson, 1979a). Both male and female titi monkeys disperse from their natal groups (Van Belle et al., 2016), but little is known about how titi monkeys choose their mates. Female titi monkeys invest more in grooming than males (Porter, 2016), male titi monkeys provide a substantial amount of paternal infant care (Wright, 1990), and both individuals invest heavily in duetting displays. In some titi monkeys (such as certain species within the genus *Plecturocebus*) there are subtle sex-related differences in the duet structure (Adret et al., 2018). Here, we aim to test the hypothesis that pulse elements of coppery titi monkey (*Plecturocebus cupreus*) duets provide information about the condition or status of the caller or pair. Duets in this species are comprised of multiple alternating call types that are emitted by both males and females. Our analysis focused on a single call type within the duet — the broadband pulse — which is comprised of stereotyped, rapidly repeating, broadband notes.

Specifically, we aimed to test whether differences in pulse elements varied by individual, pair, sex, age, weight or duration of the pair bond. We predicted that if pulse elements provide cues about caller condition, younger, heavier individuals would have longer pulses with higher pulse rate. In addition, we predicted that males would be more invested in the duetting display, given the substantial fitness costs for males associated with cuckoldry and extra-pair paternity, such that males would have higher pulse rate and longer pulses. We also predicted that there would be acoustic convergence among pair-mates, such that pulse elements of individuals within a pair would be more similar to each other than pulse elements of extra-pair individuals. Lastly, we predicted that pulse rate and duration would vary with pair bond duration. It takes at least six months for new titi monkey pairs to solidify their pair-bond, and during this time there is a marked increase in territorial behaviours (including duets; Rothwell, 2018). Therefore, we predicted that titi monkeys would invest less in the duetting display the longer they have been paired with their partner, leading to a decrease in pulse rate and duration over time.

2. Methods

2.1. Study location and subjects

All recordings of coppery titi monkey (*Plecturocebus cupreus*; hereafter titi monkey) duets were collected at the California National Primate Research Center (CNPRC), Davis, CA, USA. All study subjects were captive born. The titi monkeys were housed indoors in cages measuring 1.2 × 1.2 × 2.1 m. The room was maintained at 21°C on a 12-h light cycle with lights on from 06:00 to 18:00. Subjects were fed a diet of monkey chow, carrots, bananas, apples, and rice cereal twice a day. Water was available ad libitum and additional enrichment was provided twice a day. Subjects were housed in male-female pairs with up to three offspring. All groups were in acoustic contact with other titi monkey pairs but had minimal visual contact with animals outside their cage. This housing situation is the same as described in previous studies of this colony (Mendoza & Mason, 1986; Bales et al., 2013). Detailed demographic data has been collected for all residents of the colony since it was established.

2.2. Data collection

Titi monkey duets were recorded opportunistically each morning between 06:00 and 07:30 by trained interns and lab members for two years (March 2017 to March 2019). All animals were recorded indoors in standardized rooms. We only used recordings in which one pair was vocalizing. Thus, the background noise was standard for all recordings. We used a Marantz PMD 660 flash recorder and a Marantz Professional Audio Scope SG-5B directional condenser microphone. Recordings were made with a sampling rate of 44.1 Hz and 16-bit resolution and saved as Waveform (.wav) audio files. Subjects were recorded duetting with their pair mate. We collected all recordings noninvasively from outside each pair's cage, and less than 3 m from the calling animals. We included all calls from one duet bout per animal (mean ± SE: 5.10 ± 0.40 calls per animal; 2.77 ± 0.23 min per bout).

2.3. Acoustic analysis

All audio recordings were compared to videos of the corresponding duet bout in order to identify each calling individual. Previous authors have referred to this particular call type as a 'pump' (Robinson, 1979a), but to keep consistent with terms used in the frog (Martínez-Rivera & Gerhardt, 2008), bird (Laiolo

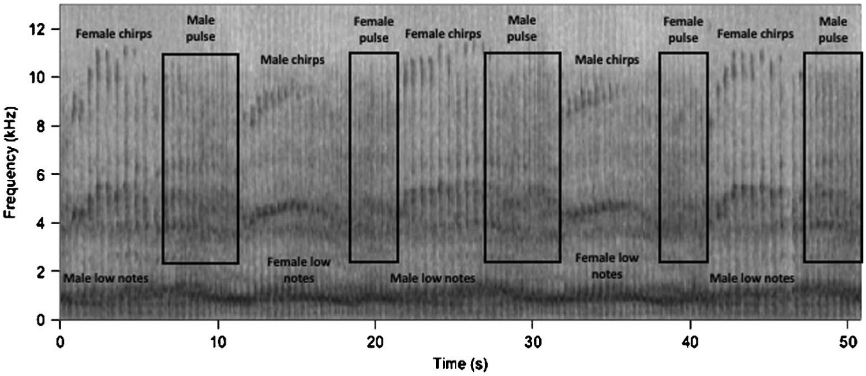

Figure 1. Representative spectrogram of a portion of a coppery titi monkey (*Plecturocebus cupreus*) duet in which pulses are highlighted. Other note types such as chirps and low notes are marked but are not analysed in this present study. Pulses occur as one individual finishes their chirp sequence, but before their duetting partner starts their chirp sequence. Both sexes sing the same repertoire of notes.

et al., 2004) and marine mammal (Mathevon et al., 2017) literature, we refer to this call type as a ‘broadband pulse’ (see Figure 1 for representative spectrogram of titi monkey duet, and Figure 2 for a representative pulse element). We only included pulses with a high signal-to-noise ratio (>10 dB) where it was clear there was only one individual emitting the pulse call type. Based on these requirements, we had to omit 81 of the original 459 pulse elements we isolated, reducing our dataset to 378 pulse elements.

We created spectrograms using Raven Pro 1.5 Sound Analysis Software (Bioacoustics Research Program 2014, Ithaca, NY, USA). We generated spectrograms with a 512-point (11.6 ms) Hann window (3 dB bandwidth = 124 Hz), with 75% overlap, and a 1024-point DFT, yielding time and frequency measurement precision of 2.9 ms and 43.1 Hz. We did not downsample the original sound files. One observer (ARL) isolated each of the pulse elements from the duet sequence and saved them as individual.wav files. Two observers (DJC and ARL) manually selected all pulse notes using the selection table feature in Raven, after confirming that inter-observer reliability was greater than 95%. For each pulse element we estimated the following parameters using the Raven selection tables: timing and number of pulse notes, along with duration of entire pulse element. To calculate pulse rate, we divided the total number of pulse notes within an element by the total duration of the element, giving a continuous measure of rate of element repetition.

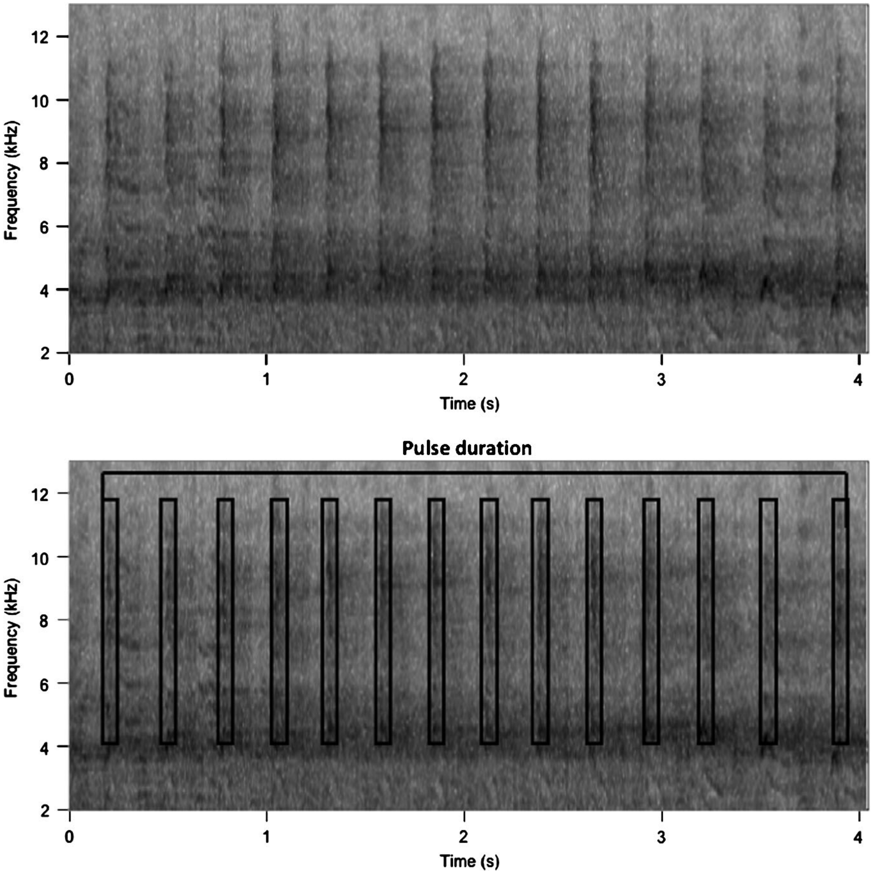

Figure 2. (Top) Representative spectrogram of a coppery titi monkey (*Plecturocebus cupreus*) pulse from the duet. (Bottom) The same representative spectrogram demonstrating how pulse notes were selected. Pulse duration was estimated by calculating the difference between the end of the last pulse and start of the first pulse. Pulse rate was calculated by dividing number of pulses by total pulse duration.

2.4. Demographic predictors of titi monkey pulse elements

To test our hypothesis and associated predictions we created a series of 10 hierarchical models predicting either pulse rate or note duration (summary of all models included in online supplementary material). Our predictor variables included sex, age (years), weight (kg), and pair length (years), and our combination of predictors reflect specific hypotheses about which variable(s) may influence titi monkey pulses. We used the ‘lme4’ package to create the hierarchical models (Bates et al., 2017) and included pair identity

Table 1.
Description of outcome and predictor variables, along with random effects, included in generalized linear models. Mean and standard error are included for continuous variables.

	Description	Mean ± SE	Range
Outcome variable			
Pulse duration	Duration of the pulse element in seconds.	2.50 ± 0.05	0.54–5.75
Pulse rate	Number of pulse notes divided by the total duration of the pulse element.	4.22 ± 0.02	2.37–5.52
Predictor variable			
Sex	Binary variable indicating whether the individual is male or female.		
Age	Age of the vocalizing individual in years.	9.78 ± 0.29	1.45–21.77
Weight	Weight of the calling individual in kilograms.	1.21 ± 0.01	0.88–1.65
Pair length	Duration of time that the individual was paired with their current duetting partner in years.	3.11 ± 0.20	0.03–15.22
Random effects			
Pair number	Pair identity		
Individual	Individual identity		

and individual identity as random effects for all models. A description of the variables included in our models, along with mean and standard deviation, is included in Table 1.

We log-transformed all of our continuous outcome and predictor variables. We checked for multi-collinearity of our predictor variables by calculating the variance inflation factor using the ‘car’ package (Fox & Weisberg, 2018). To compare models, we used an information theoretic approach based on Akaike’s Information Criterion (AICc adjusted for small sample sizes; Burnham & Anderson, 2004) using the ‘MuMIn’ package (Bartoń, 2013). We considered predictors reliable if their 95% confidence intervals did not overlap zero. We calculated a ‘pseudo R^2’ as a goodness of fit measure for our models, focusing on the conditional R^2 which can be interpreted as the amount of variance explained by both fixed and random factors using the ‘r.squaredGLMM’ function in the ‘MuMIn’ package (Bartoń, 2013).

We conducted all statistical analyses in the R programming environment (R Development Core Team, 2017).

2.5. Sources of variance in titi monkey duet pulse elements

Our dataset, like most acoustic datasets, consisted of multiple nested or hierarchical levels. For this particular dataset we had three levels, as we recorded multiple pulse elements from multiple individuals from different pairs. Each of the three levels (pulse, individual and pair) can be considered sources of variance, and to investigate variance partitioning across each level we used a Bayesian multivariate, variance components model (Clink et al., 2018). We defined our model for pulse element e, individual i, pair p as

$$\boldsymbol{Y}_{p,i,e} = \boldsymbol{a}_p + \boldsymbol{b}_i + \boldsymbol{e}_{p,i,e} \tag{1}$$

where $\boldsymbol{y}$ is the log-transformed feature vector, $\boldsymbol{a}$ is a pair-specific random intercept, $\boldsymbol{b}$ is a individual-specific random intercept, and $\boldsymbol{e}$ is a pulse-specific error term. Variance/covariance matrices at each of the three levels measure the variability in each feature, along with the covariance among features. The variance/covariance matrices for $\boldsymbol{a}$, $\boldsymbol{b}$ and $\boldsymbol{e}$ are defined, respectively, as $\boldsymbol{\Sigma}_a$, $\boldsymbol{\Sigma}_b$, and $\boldsymbol{\Sigma}_e$. We calculated intraclass correlation coefficients (ICCs) from the posterior samples of the covariance matrices $\boldsymbol{\Sigma}_a$, $\boldsymbol{\Sigma}_b$, and $\boldsymbol{\Sigma}_e$, which measure the relative contribution of each level (in this case pulse, individual and pair) to the total variance. We calculated ICCs at level l for both features (pulse rate and duration) from posterior samples of $\boldsymbol{\Sigma}_a$, $\boldsymbol{\Sigma}_b$, and $\boldsymbol{\Sigma}_e$ as:

$$\mathrm{ICC}_l = \frac{\text{Variance of feature at level } l}{\text{Total variance of feature}}.$$

ICC values range from 0 to 1, and an ICC value of 1 indicates that a particular level is an important source of variance (Merlo et al., 2005). We used the package ‘rstan’ for model implementation (Guo et al., 2016) and simulated 500 parameter samples from two Markov chains after a warmup of 4000 samples. For more detail on model development and specifications see Clink et al. (2018) and Lau et al. (2018).

2.6. Data availability

All data and R code needed to recreate our analyses is included as online supplementary material. Access to raw sound files will be provided by reasonable request to the corresponding author.

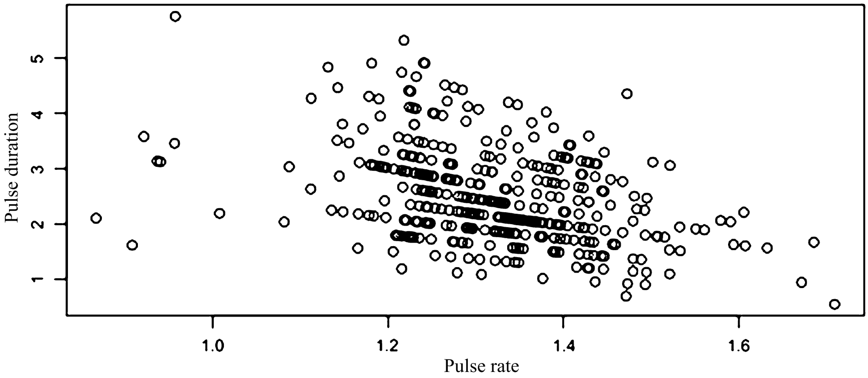

Figure 3. Correlation plots for pulse rate and duration in titi monkey duet pulse elements. See main text and Table 1 for description of how features were estimated from spectrograms of titi monkey duets.

2.7. *Ethical note*

No animals were handled in this study. This project was approved under the University of California, Davis Institutional Animal Care and Use Committee protocol No. 20834. Data collection for this project met all legal requirements of the United States and principles of the American Society of Primatologists for the ethical treatment of non-human primates.

3. Results

3.1. *Variability in titi monkey pulses*

We report the results of an analysis of 378 pulse elements from duet contributions of 74 titi monkey individuals (mean 5.12 pulses per individual, range 1–15). Pulse duration and pulse rate were negatively correlated (−0.41; Figure 3). We examined correlation among our continuous predictor variables and found that although pair length and age were positively correlated (0.57), our variance inflation factors were all below 1.5, indicating that multicollinearity of predictor variables should not influence inference from the models. We found that there was a substantial amount of inter-individual variation in pulse rate (mean ± SE = 4.22 ± 0.02) and pulse duration (mean ± SE = 2.50 ± 0.05; Figure 4).

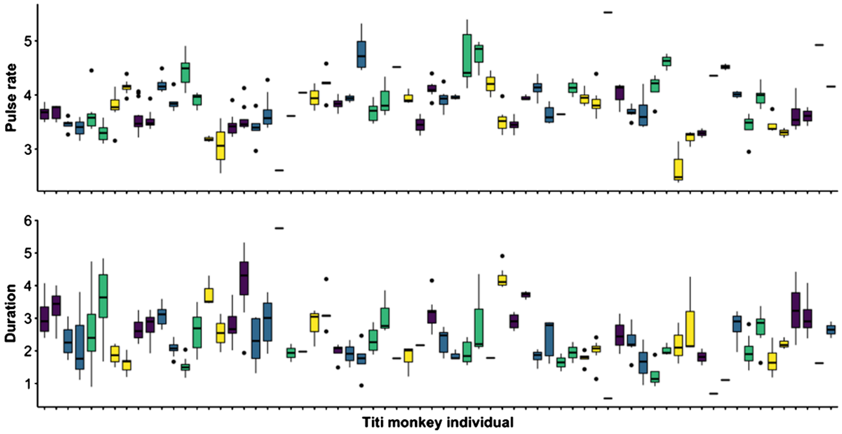

Figure 4. Boxplots of duet pulse rate and pulse duration for coppery titi monkey (*Plecturocebus cupreus*) individuals included in the study. Boxes are ordered by pair and sex, with the female of the pair on the left. Colour scheme was used for enhanced visualization of differences between nearby pairs; repeated colours across pairs are not indicative of meaningful differences. Three individuals that did not have a pair mate were removed for better visualization. Boxplots were made using the R package 'ggpubr' (Kassambara, 2018).

3.2. *Demographic predictors of titi monkey pulse elements*

Our top model for pulse rate (based on AICc comparisons) included only one predictor variable (age) and accounted for 39.5% of the model weight, while the next highest ranked model included both age and weight (ΔAICc $=$ 2.1; 14.1% of the model weight). Together, these two models account for 53% of the model weight. The third highest ranked model was the intercept only model (ΔAICc $=$ 2.3; 12.2% of the model weight), which includes random intercepts for pair and individual. Based on our top model, age had a reliably negative effect on pulse rate (estimate $= -0.027$, SE $= 0.012$; Figure 5; Table 2). The marginal R^2 for the top model for pulse rate, which represents the variance explained by our predictor variable (age) is 0.058, and the conditional R^2 for the top model is 0.795, indicating that the top model explained a substantial amount of the total variance in pulse rate.

Our top model for pulse duration included four predictor variables: age, sex, weight, and pair length, and accounted for 23.7% of the model weight. The next top model included three predictor variables: age, sex, weight, and pair length. Together these two models account for 45% of the model weight, which is substantially better than the intercept only model (ΔAICc $=$ 3.3; 4.4% of the model weight). Based on the model for pulse duration, age had a

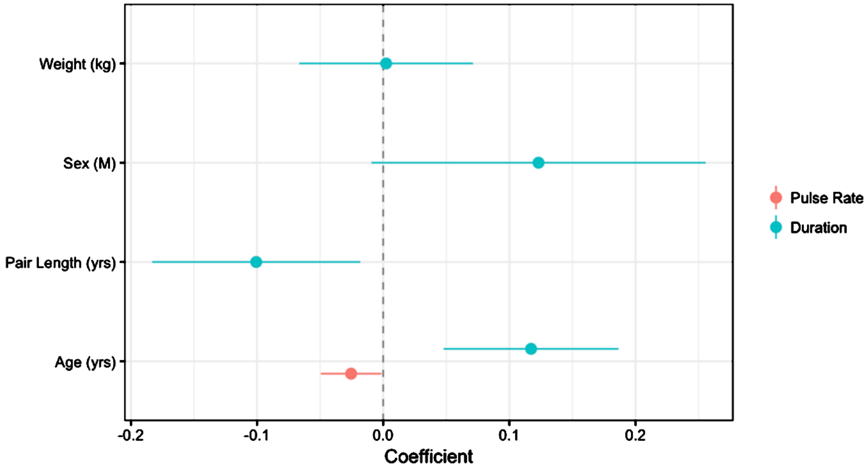

Figure 5. Coefficient estimates (including 95% confidence intervals) for top models of pulse rate and pulse duration in titi monkey duets ranked by AICc. Coefficient estimates that are less than zero indicate a lower than expected value in the outcome variable, whereas coefficient estimates that are above zero indicate a higher than expected value. Coefficients can be considered reliable if their 95% confidence intervals do not overlap zero.

reliably positive effect on pulse duration (estimate = 0.117, SE = 0.042) and pair length had a reliably negative effect (estimate = −0.100, SE = 0.050). Weight was included in the top model, but it had only weak reliability as a predictor (estimate = 0.002, SE = 0.042), and this was also the case with sex (estimate = 0.123, SE = 0.080; Figure 5; Table 2). The marginal R^2 for the top model was 0.101 and the conditional R^2 was 0.596, indicating that the top model explained a substantial amount of the variance in pulse duration.

3.3. Sources of variance in titi monkey duet pulse elements

We calculated ICCs for pulse rate and duration based on estimates of the posterior densities for each of the three levels in our dataset (pulse, individual and pair: Figure 6). We found that for pulse rate, variance at the pair-level was the most important source of variance (ICC posterior mean = 0.40; 95% posterior credibility interval = (0.14, 0.64)), and variance at the individual-level was also important (ICC posterior mean = 0.36; 95% posterior credibility interval = (0.16, 0.64)). We found that pulse rate within a duet did not exhibit a high amount of variance, as the mean ICC at the pulse-level was 0.23 (95% posterior credibility interval = (0.10, 0.31)). For pulse duration, variance at the individual- and pulse-level were both important contributors to total variance (individual ICC posterior mean = 0.48;

Table 2.
Akaike's information criterion (AICc) model comparison results, showing the top two models and the null model for each outcome variable (pulse rate and pulse duration).

Outcome model	Log-likelihood	AICc	dAICc	df	Weight	Marginal R^2	Conditional R^2
Pulse rate							
Age	−180.23	370.623	0.000	5	0.395	0.058	0.796
Age × weight	−180.23	372.686	2.063	6	0.141	0.058	0.796
Intercept only	−182.43	372.973	2.350	4	0.122	0.000	0.798
Pulse duration							
Sex × age × weight × pair length	−52.16	120.72	0.000	8	0.237	0.100	0.625
Age × weight × pair length	−53.31	120.94	0.211	7	0.213	0.084	0.627
Intercept only	−57.97	124.06	3.335	4	0.044	0.000	0.630

95% posterior credibility interval = (0.26, 0.67); pulse ICC posterior mean = 0.46; 95% posterior credibility interval = (0.16, 0.67)), whereas pair-level variance was not an important contributor to total variance (ICC posterior mean = 0.06; 95% posterior credibility interval = (0.01, 0.22)). Corroborating our finding that pair-level variance was the most important source of variance in pulse rate, we found that there was a strong correlation between mean male and female pulse rate within a particular duetting pair (Figure 7).

4. Discussion

Our analysis of pulse elements from the duets of 74 titi monkey individuals revealed substantial inter-individual variation in pulse rate and pulse duration. Age of the duetting individual was a reliable predictor of pulse rate, with older monkeys exhibiting slower pulse rates. Age and pair length were reliable predictors of pulse duration. Older monkey pulse elements were of longer duration, and although age and pair length were positively correlated (0.57), pair length had the opposite effect on duration, meaning animals that were paired longer had pulse elements of shorter duration. Contrary to previous reports of subtle sex differences in duets of the moloch lineage (Adret et al., 2018), we did not find evidence of substantial sex differences in pulse elements. A portion of the variation in our features was not explained by our predictor variables, and we were interested to see how variance in pulse

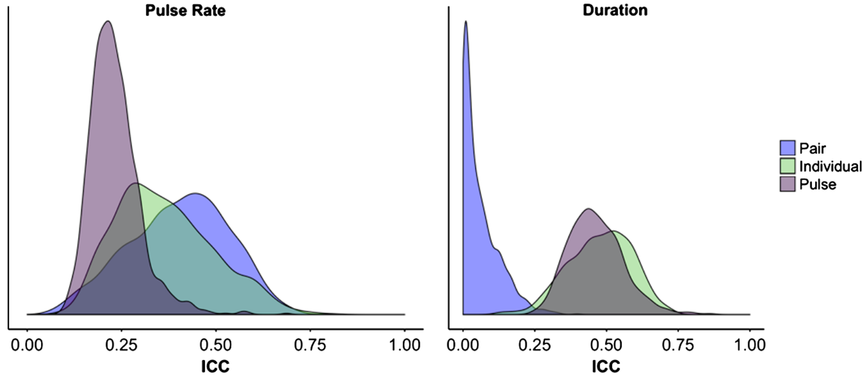

Figure 6. Posterior density estimates of intra-class correlation coefficients (ICCs) for each of the three levels in our dataset (pulse, individual, and pair) for pulse rate and pulse duration. For pulse rate, pair-level variance was the most important contributor to total variance, and for pulse duration individual-level variance was most important. The y-axis refers to density and is not shown; only the relative densities for each feature matter.

rate and duration was partitioned across our three levels of analysis: pairs, individuals and pulse elements. We found that pair-level variance was the most important source of variance for pulse rate, providing evidence for vocal convergence among pair mates in this feature, and that individual-level variance was most important for pulse duration. Our findings contribute to the growing body of evidence indicating that nonhuman primate vocaliza-

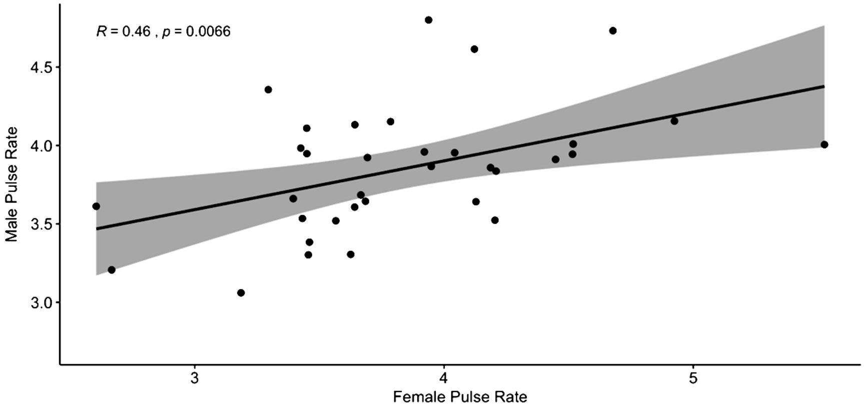

Figure 7. There was a strong positive correlation in mean pulse rate between male and female duetting partners. Each point represents the mean pulse rate for the male and female within a pair ($N = 34$). Three individuals included in the study did not have a pair mate and were not included in the figure.

tions — rather than being innate and inflexible — exhibit a high degree of plasticity, and support the idea that behavioural environment plays an important role in shaping adult primate vocalizations (Bergman et al., 2019).

4.1. Age related changes in pulse elements

We found that as titi monkeys get older, their pulse rate decreases. This could be due to a decline in animal quality with age — if pulse rate is indeed an honest indicator of animal status — and quickly repeated elements are difficult to produce (Byers et al., 2010). Our results are consistent with findings in lar gibbons, where females exhibit age-related decline in call performance, with older females having lower frequency calls and a higher ratio of rest duration to note duration (Terleph et al., 2016). Although we found that pulse rate decreases with age, we found that the opposite pattern occurs with duration, wherein duration of pulses increased with age. It is possible that as animals get older, they may not be able to produce pulses at as high of a rate but call for a longer duration of time to account for this decreased repetition rate. Pulse elements may be constrained by energetic output (Ophir et al., 2010) and changes in physiology of animals as they age. In addition to physiological effects, covariance between pulse elements and age may be related to motivation of calling animals. For example, in birds it has been shown that rate of note repetition increases in aggressive contexts (DuBois et al., 2009; Linhart et al., 2013; Furutani et al., 2018). Humans articulate faster when they are scared or under stress (Scherer, 1986), and this is also the same for chimpanzees (Siebert & Parr, 2003). Therefore, it is possible that younger titi monkeys are in a different emotional state than older monkeys, leading to faster pulses within their duets.

4.2. Pair bond duration and evidence for vocal convergence

We found a negative relationship between pair bond length and duration of duet pulse elements. In the lab, partner preference tests have demonstrated that it takes around six months for the titi monkey pair bond to be considered fully formed (Rothwell, 2018). This could influence pulse duration, as mates often participate in costly mate-guarding (Komdeur, 2001; Brotherton & Komers, 2003) and territorial behaviors (including vocal duetting) as they solidify their pair bond. Additionally, titi monkeys are often actively mating and potentially gestating during the first six months of their pair bond (Valeggia et al., 1999). This may also lead to increased territorial behaviours.

It is unclear how (and if) these results would translate to wild populations in which animals are able to choose their partners, and studies on the link between pair bond length and pulse duration in wild titi monkeys will be informative.

We also found evidence for vocal convergence among pair mates in pulse rate, as pair-level variance was the most important source of variance for this feature, and there was a correlation between mean pulse rate of males and females within a particular pair. Interestingly, we did not find that pair-bond duration had an effect on pulse rate. The mean duration of pair bond in our dataset was about 3 years, and it may be that pairs converge in pulse rate early on in pair-bond formation, so we were not able to detect an effect in our dataset. Vocal convergence among group members has been shown across the Primate Order, in tarsiers (Clink et al., 2019), New World monkeys (this study; Snowdon & Elowson, 1999), Old World monkeys (Lemasson et al., 2011) and great apes (Mitani & Gros-Louis, 1998; Levréro et al., 2019). In budgerigars (*Melopsittacus undulates*), a small species of parrot, male vocal imitation of female contact calls led to convergence, and the authors posited that vocal convergence is related to pair bond formation and/or maintenance in this species (Hile et al., 2000). It is possible that vocal convergence in titi monkey duets is similarly related to pair-bond formation, and that development of a common set of duet characteristics communicates to neighbouring conspecifics that a pair-bond has been established (Snowdon & Elowson, 1999). As titi monkey pairs in our study were formed artificially and in the absence of assortative mating, wherein individuals may choose to form pairs with individuals exhibiting similar duet structure, our results indicate that vocal convergence is a consequence of vocal plasticity wherein one or both individuals modify the structure of their calls to match their partner. Future longitudinal studies of newly paired titi monkeys will help improve understanding of the mechanisms and time frame in which vocal convergence in pulse rate occurs.

4.3. Sex-specificity

Primate duets exhibit varying levels of sex-specificity, with duets of gibbons and tarsiers exhibiting markedly distinct male and female contributions, whereas indri and titi monkey duets exhibit relatively low levels of sex-specificity. Indri duets and choruses exhibit a great degree of overlap among singers, with low levels of sex-specificity, but the dominant male and female of the group overlap more than other members (Gamba et al., 2016). Male indris also have higher frequency contributions than females, but there are not differences among age classes. While the moloch lineage of titi monkeys (in which coppery titi monkeys are currently classified) has shown the potential for sex differences in duet vocalizations (Aldret et al., 2018), in this species, both sexes have the same vocal repertoire (Robinson, 1979b). One study of this species did find potential sex differences in the frequency of bellows (low frequency notes; Robison, 1979b), but another study found no sex differences in frequency (Muller & Anzenberger, 2002). We did not find evidence of substantial sex differences in pulse rate, or pulse duration, and it is unclear whether sex-specificity in this species varies by call type, or if previously documented sex differences are related to methods used for analysis.

Previous studies investigating the link between rate of element repetition and caller condition have focused on male songbirds (Drăgănoiu et al., 2002; Ballentine et al., 2004; de Kort et al., 2009; Cramer, 2013) or male singing mice (Pasch et al., 2011). Most of these focus on variation in element repetition in the context of female preference. We show that for titi monkey pulses, there are no sex-differences in either pulse rate or pulse duration. We also show that pulse rate varies with age, regardless of sex. Therefore, pulse rate may provide a reliable cue of age (and therefore caller condition) for both male and female titi monkeys. In addition, as there are few quantifiable sex differences in titi monkey duets, it appears that both male and female contributions serve a similar function (Riebel et al., 2019).

4.4. Implications for studies on captive versus wild populations

While the vocalizations analysed here are species-specific and comparable to those recorded in wild populations of the same species (Robinson, 1979b), there are notable and obvious differences in the socioecology of this present population and their conspecifics in the wild. Most notably, the intergroup spacing between titi monkey pairs is significantly altered in the captive environment. In the wild, titi monkeys occupy distinct territories of approximately 3 to 4 hectares (Robinson, 1979b) that they actively defend from conspecifics. In captivity, these territorial, vocal monkeys are housed in indoor rooms in acoustic contact with up to 15 other pairs of monkeys. In the wild, titis encounter far fewer conspecifics in or near their territory. This difference in population density may influence rates of cultural transmission or vocal learning.

Similarly, all titi monkeys in the *Plecturocebus cupreus* colony are paired by management teams to ensure inbreeding prevention and success of the colony. Thus, animals do not choose their pair mates. Rather, pair mates are selected by researchers. This artificial pairing paradigm, while necessary in a closed, captive colony, is different from how individuals choose their mates in the wild. As vocal duetting is a highly coordinated behaviour between pair mates, mate choice and pair bond quality may impact duet variability. In addition, we had to eliminate pulses from the present analysis that were of low-quality or had overlapping individuals, so we may not have captured the full scope of variance in the titi monkey duet pulse element. Beyond these challenges, captive-housed primates present the opportunity to answer questions likely unanswerable in wild populations. Because this colony of titi monkeys has been captive housed for eight generations, we have detailed demographic information about a substantial number of animals that would be nearly impossible to acquire through wild studies without significant investment of resources and man-power.

4.5. Future directions

We have shown that titi monkey pulse elements vary with age and duration of pair bond, but it is unclear whether variation in pulse elements confers any information to listening conspecifics about signaller quality. A natural next step in identifying the importance of the variability we see in titi monkey pulses would be to conduct playback experiments to see if titi monkeys alter their behaviour in response to recordings of animals with varying pulse rate and duration. The titi monkeys' behavioural response to these playbacks will elucidate whether or not titi monkeys can perceive variability in different pulses.

Further, a substantial amount of the variance in pulse elements was explained by individual identity, meaning there are likely factors other than age and pair mate that influence individual variation in pulse elements. In particular, we found that pulse duration exhibited substantial inter-individual variation, whereas pulse rate varied consistently across pairs. It is possible that a single call-type within the duets (the pulse element) encodes information regarding both individual and pair identity (Snowdon & Elowson, 1999). Other species of primates have demonstrated considerable individuality in their vocalizations (Mitani et al., 1996; Lameira & Wich, 2008; Leliveld et al., 2011; Clink et al., 2017), and we expect to find a similar pattern in coppery titi monkeys. A pattern of individuality would be intuitive for reasons described above: pulses are likely perceived by neighbouring pairs and one's mate and thus confer information to conspecifics about caller status. If pulses are individually identifiable, a titi monkey should only respond aggressively to unknown pulses (known as the 'dear enemy effect'; Lesbarrères & Lodé, 2002; Bee, 2003; Radford, 2005; Papworth et al., 2009; Moser-Purdy et al., 2018). Future studies into individual variability in titi monkey duets, along with playbacks of simulated territorial intrusions to determine if conspecifics pick up on individually-distinct acoustic cues, will be informative.

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