Nineteen Years of Consistently Positive and Strong Female Mate Preferences despite Individual Variation

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ABSTRACT: Sexual selection driven by mate choice has generated some of the most astounding diversity in nature, suggesting that population-level preferences should be strong and consistent over many generations. On the other hand, mating preferences are among the least repeatable components of an individual animal's phenotype, suggesting that consistency should be low across an animal's lifetime. Despite decades of intensive study of sexual selection, there is almost no information about the strength and consistency of preferences across many years. In this study, we present the results of more than 5,000 mate choice tests with a species of wild frog conducted over 19 consecutive years. Results show that preferences are positive and strong and vary little across years. This consistency occurs despite the fact that there are substantial differences among females in their strength of preference. We also suggest that mate preferences in populations that are primarily the result of sensory exploitation might be more stable over time than preferences that are primarily involved in assessing male quality.

Keywords: túngara frog, Physalaemus pustulosus, sexual selection, mate choice, mating call.

> In the first place, it seems quite incredible, without direct evidence on the point, that a large majority of the females of any species, over the whole area of its range *and for many successive generations*, should agree in being pleased by the same particular kind of variation. (Wallace 1876; emphasis added)

Introduction

Mate choice and the preference rules underlying it have been of both interest and controversy since Darwin (1871) fleshed

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out his theory of sexual selection. Unlike selection forces generated by the environment, mate choice is both an agent and a target of selection. As an agent of selection it can drive the evolution of elaborate traits in members of the opposite sex, and as a target of selection it can influence the fitness of individuals exercising mate choice (Kirkpatrick and Ryan 1991). We follow Kirkpatrick et al. (2006) and use the term "mate choice" as the outcome of an animal choosing to mate with one individual versus others and the term "preference" as the rules that underlie those choices.

Sexual selection, in general but especially sexual selection by female mate choice, has generated controversy dating back to the initial disagreement between Darwin and Wallace (Richards 2017) apparent in the epigraph. Wallace was not the only one to doubt the ubiquity of female mate choice. Julian Huxley (1938, p. 417) stated that "competition between males for mates, accompanied by any form of female choice, is not the common phenomenon postulated by Darwin." More recently, Roughgarden et al. (2006, p. 965) asserted that "sexual selection is always mistaken, even where gender roles superficially match the Darwinian templates"; they instead emphasized the role of cooperation over that of Darwin's notion of sexual conflict. On the other hand, many if not most biologists have argued that there is ample evidence to demonstrate that female choice exists and is common (Andersson 1994; Prum 2017; Rosenthal 2017; Ryan 2018). Nevertheless, it is not known whether it is consistent over many generations, as Wallace (1876) doubted.

Why should it matter whether mate choice is consistent or inconsistent across generations? Lehtonen et al. (2010, p. 21) state that for male sexual ornaments to evolve "females need to prefer an extreme expression of the male trait, and the preferences themselves should be concordant among individuals within the population and remain fairly consistent across time (Mead and Arnold 2004)." They further posit that there is no reason to expect female preferences to be

more evolutionarily stable than male signal traits (Bakker et al. 1999; Qvarnström 2001). But these same authors point out that very little is known about the temporal consistency of female preferences (see also Jennions and Petrie 1997; Qvarnström 2001).

It is well known, however, that natural selection can vary across time. In a classic study by Gibbs and Grant (1987), fluctuating rainfall across years influenced seed-type availability, which in turn influenced variation in beak shape in Galapagos finches; different beak shapes are adapted for different food types. In these and many other examples of natural selection (e.g., Endler 1986), there is no feedback between the agent of selection—in this example, rainfall—and the target of selection, the birds' beaks.

On the other hand, there is ample opportunity for a feedback loop in mate choice as well as in other instances of social selection more generally (West-Eberhard 1979). This feedback loop could induce variation in those male traits that are targeted by female preferences and thus cause preferences to vary across time. Building on the Galapagos finches example, beak shape can act as a species recognition cue, and Grant and Grant (1997) suggested that it can also contribute to premating isolation (see also Price 2008; Grant and Grant 2018). Thus, it might be expected that females would preferentially mate with males with beaks better adapted for the current and local food supply. The female preference would act as an agent of selection on beak shape, but the preference would also be a target of selection as the beak shape of her partner could influence her reproductive success. As local food conditions change, so does beak shape as well as the preferences for beak shape. In such a scenario, referred to as the ecological crossover hypothesis by Greenfield and Rodriguez (2004), different male traits will be more indicative of male condition in different environments, and this should favor plasticity in mate choice. Botero and Rubenstein (2012) reviewed a substantial body of literature on mating systems in birds and concluded that across species, environmental variability did favor plasticity in female mate choice. We note that the data analyzed in that study were cross-species comparisons and not longitudinal comparisons within species.

Would variation in mating preferences across generations be surprising? No. We do know that, in general, behavior can be a quite variable aspect of the phenotype, and this might be especially true of mate choice behavior, as surveys of repeatability have shown. (Repeatability is a statistical measure of variability that compares the within- and among-individual variation in a population: $r = s_A^2/s^2 + s_A^2$, where s_A^2 is the variance among individuals and s^2 is the variance within individuals; Bell et al. 2009.) In a survey of 759 estimates of repeatability in behavior representing 98 species, mate choice was one of the best studied but least repeatable of all behaviors (Bell et al. 2009). Similarly, Rosenthal (2017) reviewed 192 studies of repeatability of mate choice and showed that the average repeatability for major taxonomic groups was usually less than 0.3, much lower than the repeatability for many other behaviors summarized by Bell and colleagues.

That mate choice is often inconsistent within individuals has suggested to some that sexual selection by mate choice might also be inconsistent across generations (e.g., see Iwasa and Pomiankowski 1995; Gosden and Svensson 2008; Kasumovic et al. 2008). However, we know little about the consistency of mate preferences across more than just a few generations (Rosenthal 2017) nearly 150 years after Wallace challenged Darwin's theory of sexual selection by female mate choice because he could not imagine that it was consistent.

Here we report the results of more than 5,000 preference tests for two mating call types, simple and complex, in a frog over a period of 19 years. As these frogs appear to live only 1 year in the wild and are sexually mature within a year of their birth (Ryan 1985), this is equivalent to at least 19 generations. We addressed two questions: How consistent is the direction and strength of preference for complex calls over simple calls across years? And is there any evidence for substantial variation among females in this preference, which could contribute to a lack of consistent preferences among years?

The System

Preference for complex calls by túngara frogs is among the better documented examples of sexual selection by female choice (Ryan 1980, 1983, 2011*b*; Rand and Ryan 1981; Ryan and Rand 1990, 1993*a*, 1993*b*; Rand et al. 1992; Ryan and Keddy-Hector 1992; Wilczynski et al. 1995, 1999; Kime et al. 1998; Farris et al. 2002; Lynch et al. 2005, 2006; Phelps et al. 2006; Bernal et al. 2009; Akre and Ryan 2010*a*, 2010*b*; Baugh and Ryan 2010*a*, 2010*b*, 2010*c*, 2010*d*, 2011; Akre et al. 2011; Dawson and Ryan 2012; Taylor and Ryan 2013; Baugh et al. 2018), and perhaps the first experimental demonstration that variation in courtship displays within a species influences female mate preferences (Ryan 1980). This preference has been documented using a vast array of natural, natural-manipulated, and synthetic calls.

Túngara frogs produce a mating call with two components. All calls begin with a whine, which males can produce in isolation (simple call) or to which they can add one to seven short bursts of sound called chucks (complex calls; fig. 1). Males in all 30 populations sampled along a 5,000-km transect throughout the species' range produced both simple and complex calls, although there are a number of differences in the acoustic details of the call among populations as well as among males in the same population (Ryan et al. 1996).

The túngara frog's whine is both necessary and sufficient for species recognition, and female frogs (Ryan 2011*b*) as well as predatory bats (Tuttle and Ryan 1981) and parasitic midges (Bernal et al. 2006) prefer complex calls to simple calls. In ad-
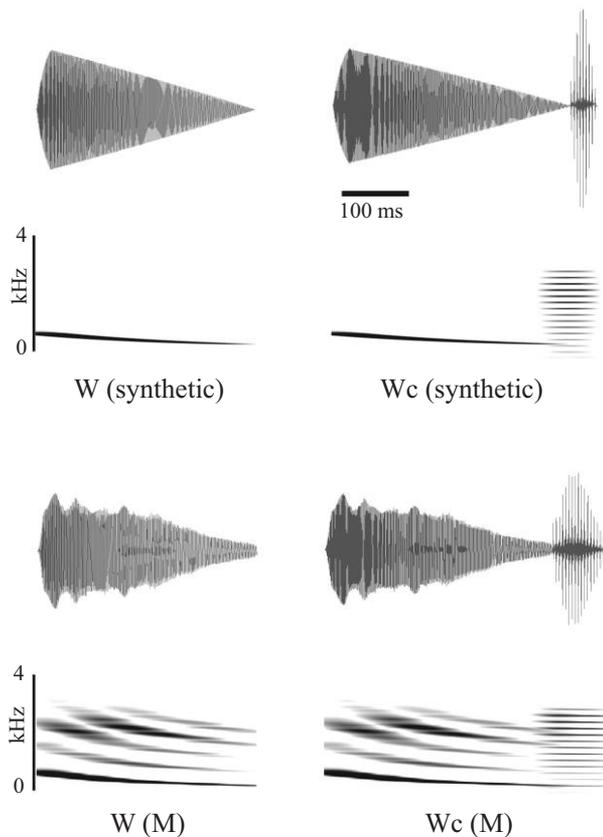
Figure 1: Oscillograms (*top*) and spectrograms (*bottom*) of a synthetic and natural whine ("W") and whine-chuck ("Wc"). The natural calls are labeled "M," the population mean call from Ryan and Rand (2003).

dition, female frogs and frog-eating bats also prefer calls with more chucks to calls with fewer chucks (Akre et al. 2011). Nonetheless, 83% of the calls recorded in natural choruses are either a whine or a whine with one chuck (Bernal et al. 2007). As there is little energetic cost to adding chucks (Bucher et al. 1982), we surmise that the reluctance of males to add chucks, despite the mating advantage of doing so, is due to the high cost of predation (Ryan 2011*b*). Despite the importance of sexual selection by female choice in driving the evolution of this sexual communication system, we know little about how the strength of these female preferences vary across years or among females within years.

Methods

Phonotaxis

We measured preferences for simple calls versus complex calls in reproductively receptive adult female túngara frogs captured from the field in and around Gamboa, Panama, between June and August each year from 1997 to 2015 inclusive. Females were captured in amplexus in early evening,

usually between 1900 and 2000 hours, and then tested that same night/morning, usually between 2000 and 0400 hours. After all testing was completed the females were released together with their male partners at the site where they were collected. To avoid retesting, females were given a unique toe-clip combination following Beaupre et al. (2004). Protocols for collecting and testing frogs were approved by the Institutional Animal Care and Use Committees of the University of Texas at Austin and the Smithsonian Tropical Research Institute. The Autoridad Nacional del Ambiente approved scientific permits in the Republic of Panama.

Females were tested for their preference between a synthetic whine versus a synthetic whine-chuck. The same synthetic calls were used every year (fig. 1; Rand et al. 1992). As noted above, the preference for complex calls versus simple calls has been well documented using a variety of natural, natural-manipulated, and synthetic calls. In this set of studies we employed a single pair of stimuli to be used as a benchmark against which we measured variation in female preferences. Thus, variation in the response of females could be contributed to the females themselves rather than to variation in the stimuli with which they were tested. The only difference between this simple and complex call is a synthetic chuck added to that same synthetic whine. This protocol allows us to control for all other acoustic variables that could covary with the complexity of natural calls.

For each test, a female was placed under a cone in the center of a 2.7 × 1.8-m Acoustic Systems sound chamber in our laboratory at the Smithsonian Tropical Research Institute facilities in Gamboa. Two speakers at equal distance from the cone (1.35 m) and opposite one another broadcast the acoustic stimuli antiphonally. Stimuli were switched between speakers after each test. Stimulus amplitude was adjusted such that the whine of each stimulus was 82 dB SPL (re. 20 μPa) in the center of the arena. Stimulus amplitude was measured with peak amplitude and flat weighting settings on a Gen-Rad 1982 SPL meter (General Radio Corporation, West Concord, MA). An infrared light illuminated the chamber, and an infrared-sensitive video camera projected an image of the chamber to a monitor outside the chamber, where female activity was scored.

Females were restrained under the cone while the speakers played for 3 min. At this time, we raised the cone from outside the chamber and the speakers continued playing. The female could then respond to the stimuli by moving to a speaker. We scored the female as making a choice when she approached to within 10 cm of the speaker. If she did not move from the center of the chamber within 5 min of raising the funnel, if she remained stationary for 2 min at any point after leaving the center of the chamber, or if she did not choose a speaker within 15 min, the test was scored as no choice. "No-choice" data were not included in the analyses, as many factors could contribute to lack of motivation

to choose, especially hormone levels at the time of testing (Lynch et al. 2005).

Testing the preference for whine versus whine-chuck was often used as a screening test to determine whether females were motivated to respond in subsequent phonotaxis experiments. Thus, females were tested a variable number of times. Data have been deposited in the Dryad Digital Repository (https://dx.doi.org/10.5061/dryad.6c13n16; Ryan et al. 2019).

Statistics

We used a generalized linear random effects modeling strategy with logit link function to estimate the variation in whine-chuck preference rates across both time (year) and individual. This strategy models the probability p_{it} that frog i tested in year t will prefer the whine-chuck call by

$$p_{it} = \frac{1}{1 + \exp(-\mu - f_i - y_t)}, \tag{1}$$

where the latent parameters f_i and y_t are modeled by random variables F_i and Y_t, respectively, with

$$\begin{aligned} \text{Cov}[F_i, F_j] &= \sigma^2 \delta_{ij}, \\ \text{Cov}[Y_t, Y_u] &= \tau^2 \delta_{tu}, \\ \text{Cov}[F_i, Y_t] &= 0 \end{aligned} \tag{2}$$

(δ_{ij} is the Kronecker delta, equal to 1 if $i = j$ and 0 otherwise). We used the function glmer from the R package lme4 to fit this model and to obtain maximum likelihood estimates.

Given the estimates of the parameters $\hat{\mu}, \hat{\sigma}, \hat{\tau}$ thus obtained, we calculate the means and variances of the preference rates P_{it} in terms of the moments

$$\begin{aligned} \mathbb{E}[P_{it}^n | \hat{\mu}, F_i = f_i, \hat{\tau}] &= \int_{-\infty}^{\infty} \left[\frac{1}{1 + \exp(-\hat{\mu} - f_i - y_t)} \right]^n \\ &\times \mathbb{P}[Y_t = y_t] \, dy_t, \end{aligned} \tag{3}$$

$$\begin{aligned} \mathbb{E}[P_{it}^n | \hat{\mu}, \hat{\sigma}, Y_t = y_t] &= \int_{-\infty}^{\infty} \left[\frac{1}{1 + \exp(-\hat{\mu} - f_i - y_t)} \right]^n \\ &\times \mathbb{P}[F_i = f_i] \, df_i, \end{aligned} \tag{4}$$

$$\begin{aligned} \mathbb{E}[P_{it}^n | \hat{\mu}, \hat{\sigma}, \hat{\tau}] &= \int_{-\infty}^{\infty} \left[\frac{1}{1 + \exp(-\hat{\mu} - f_i - y_t)} \right]^n \\ &\times \mathbb{P}[F_i = f_i] \mathbb{P}[Y_t = y_t] \, df_i \, dy_t, \end{aligned} \tag{5}$$

where $F_i \sim \mathcal{N}(0, \hat{\sigma}^2)$ and $Y_t \sim \mathcal{N}(0, \hat{\tau}^2)$. The overall mean of the random variable P_{it} representing the preference rate of a frog i in year t is then given by equation (5) with $n = 1$, while the corresponding overall variance is given by

$$\mathbb{V}[P_{it} | \hat{\mu}, \hat{\sigma}, \hat{\tau}] = \mathbb{E}[P_{it}^2 | \hat{\mu}, \hat{\sigma}, \hat{\tau}] - \mathbb{E}[P_{it}^1 | \hat{\mu}, \hat{\sigma}, \hat{\tau}]^2. \tag{6}$$

We characterize the relative contributions of year-to-year variance and frog-to-frog variance by

$$\begin{aligned} \mathbb{V}[P_{it} | \hat{\mu}, F_i = 0, \hat{\tau}] &= \mathbb{E}[P_{it}^2 | \hat{\mu}, F_i = 0, \hat{\tau}] \\ &\quad - \mathbb{E}[P_{it}^1 | \hat{\mu}, F_i = 0, \hat{\tau}]^2. \end{aligned} \tag{7}$$

$$\begin{aligned} \mathbb{V}[P_{it} | \hat{\mu}, \hat{\sigma}, Y_t = 0] &= \mathbb{E}[P_{it}^2 | \hat{\mu}, \hat{\sigma}, Y_t = 0] \\ &\quad - \mathbb{E}[P_{it}^1 | \hat{\mu}, \hat{\sigma}, Y_t = 0]^2. \end{aligned} \tag{8}$$

Here we have made the estimates for the contribution of each random effect with the value of the other random effect terms held at its average value (zero, by definition).

Results

We conducted 5,123 phonotaxis tests with 2,464 females. Sample sizes of tests over the 19 years ranged from 43 to 714, and in 16 of the 19 years sample sizes were 100 tests or greater (fig. 2): 1,379 females were tested once, 7 were tested between seven and nine times, while the remainder, 1,078, were tested between two and six times (fig. 3).

Summing all of the data across years, females preferred the whine-chuck to the whine in 4,404 of the 5,123 preference tests, while in only 719 tests did females choose the whine (cumulative binomial $P < .00001$). Thus, the strength of preference for the complex call was 0.8597. If we consider only the first test with each female, 2,116 of the 2,464 females tested preferred the whine-chuck to the whine (cumulative binomial $P < .00001$), a strength of preference of 0.8588, nearly identical to the strength of preference when multiple tests by females are taken into account.

Our generalized linear model obtained maximum likelihood estimates $\hat{\mu} = 2.1$ for the mean parameter in equation (1) as well as $\hat{\sigma}^2 = 0.79$ and $\hat{\tau}^2 = 0.11$ for the variance parameters in equation (2). These parameters correspond to estimates of a within-year standard deviation of 0.10 across frogs and a much lower standard deviation of 0.033 across years in the preference rates p_{it} (with an average preference rate of 0.86).

We compared model (1) to three simpler models:

$$p_{it} = \frac{1}{1 + \exp(-\mu - y_t)}, \tag{9}$$

$$p_{it} = \frac{1}{1 + \exp(-\mu - f_i)}, \tag{10}$$

$$p_{it} = \frac{1}{1 + \exp(-\mu)} = p_0. \tag{11}$$

Equation (9) simplifies equation (1) by assuming that there is no variation in preference rates between individual frogs. On the other hand, equation (10) retains the frog-to-frog variation but assumes no variation between years. Finally, equa-
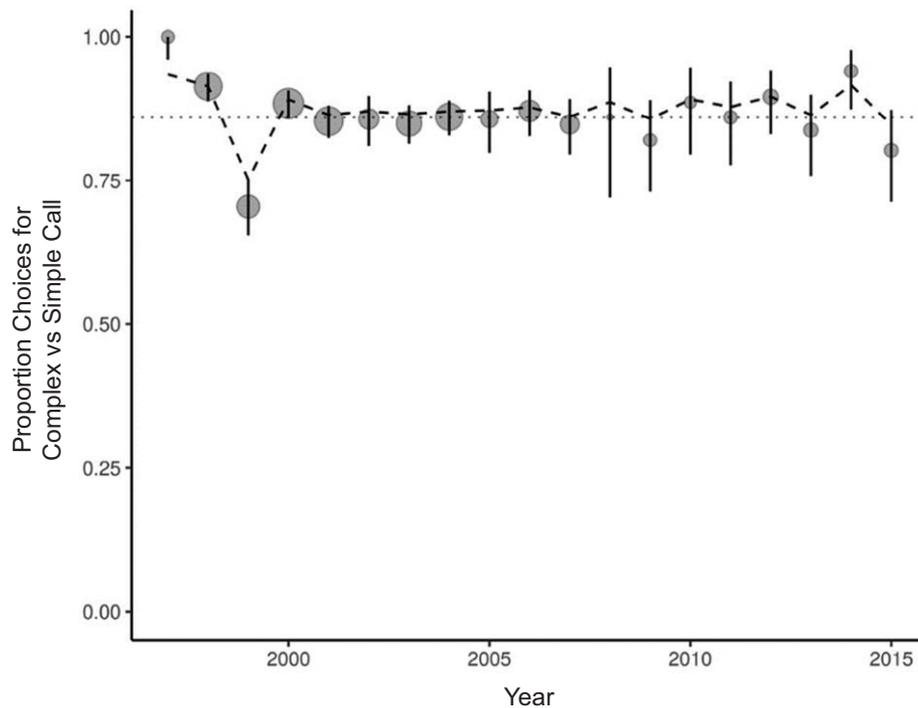
Figure 2: Proportion of female choices for the complex over the simple call (with 95% Clopper-Pearson binomial confidence intervals). Sample sizes are indicated by the size of the circle and range from 43 to 714. The dotted horizontal line represents the average rate of preference for the complex over the simple call (0.86). Finally, the estimated preference rates associated with each year according to the generalized linear random effects model equation (1) are presented as the jagged dashed line.

tion (11) omits both sources of variation, thus collapsing down to a simple single-parameter binomial model.

We apply the Akaike information criterion (AIC; Akaike 1974) to estimate the relative quality with which each of these models fits the data as quantified by Kullback-Leibler divergence from the (unknown) true data generation process. Lower AIC values indicate better model fits, with the quantity $\exp([1/2]\Delta\text{AIC})$ approximating the odds that the lower AIC model is actually superior to the higher AIC model in a head-to-head comparison (Burnham and Anderson 2003); for example, $\Delta\text{AIC} = 6$ indicates that the lower AIC model is about 20 times more likely to be preferable than the higher AIC model, while for $\Delta\text{AIC} > 30$ the odds exceed $20^5 = 3.2$ million to 1. Table 1 presents the variance estimates and AIC values for each of these models: the full model equation (1) is preferred by AIC, followed closely by the reduced model equation (10) accounting for variation among frogs but neglecting variation among years. Model (1)–estimated variance among frogs is much larger than the estimated variance among years (roughly corresponding to a ±10% preference rate from frog to frog and a ±3% preference rate from year to year; see eqq. [7] and [8] in "Methods" for details). Inspection of figure 2 suggests that the variation among years might be driven by data solely from 1999. Excluding these data yields a model in which all variation in preference rate is es-timated to be frog to frog (±13%), with zero variation associated with year. There are no reasons, however, to suspect that the data from 1999 are aberrant in any way. In general, our results show that there is minor variation among years in the strength of female preference for the complex call over the simple call despite the fact that there is substantial variation among females within years.

Discussion

Despite intense interest in sexual selection and mate choice over the past 50 years, there is an astounding lack of information on how consistent mating preferences are across years (Rosenthal 2017). This is despite a recent emphasis throughout science on the importance of replicability of studies (Ryan 2011*a*; McNutt 2014; Fraser et al. 2018).

Numerous studies have shown that mating preferences within individuals are often inconsistent (summarized in Bell et al. 2009; Rosenthal 2017). There are only a few studies that examine patterns of mate choice across years. Qvarnström et al. (2006) analyzed a large multiyear data set in which mate choice by collared flycatchers was inferred by phenotype of the male partner. These data were then used to infer heritabilities of female preferences and male phenotype. But they do not report variation among years. In another study
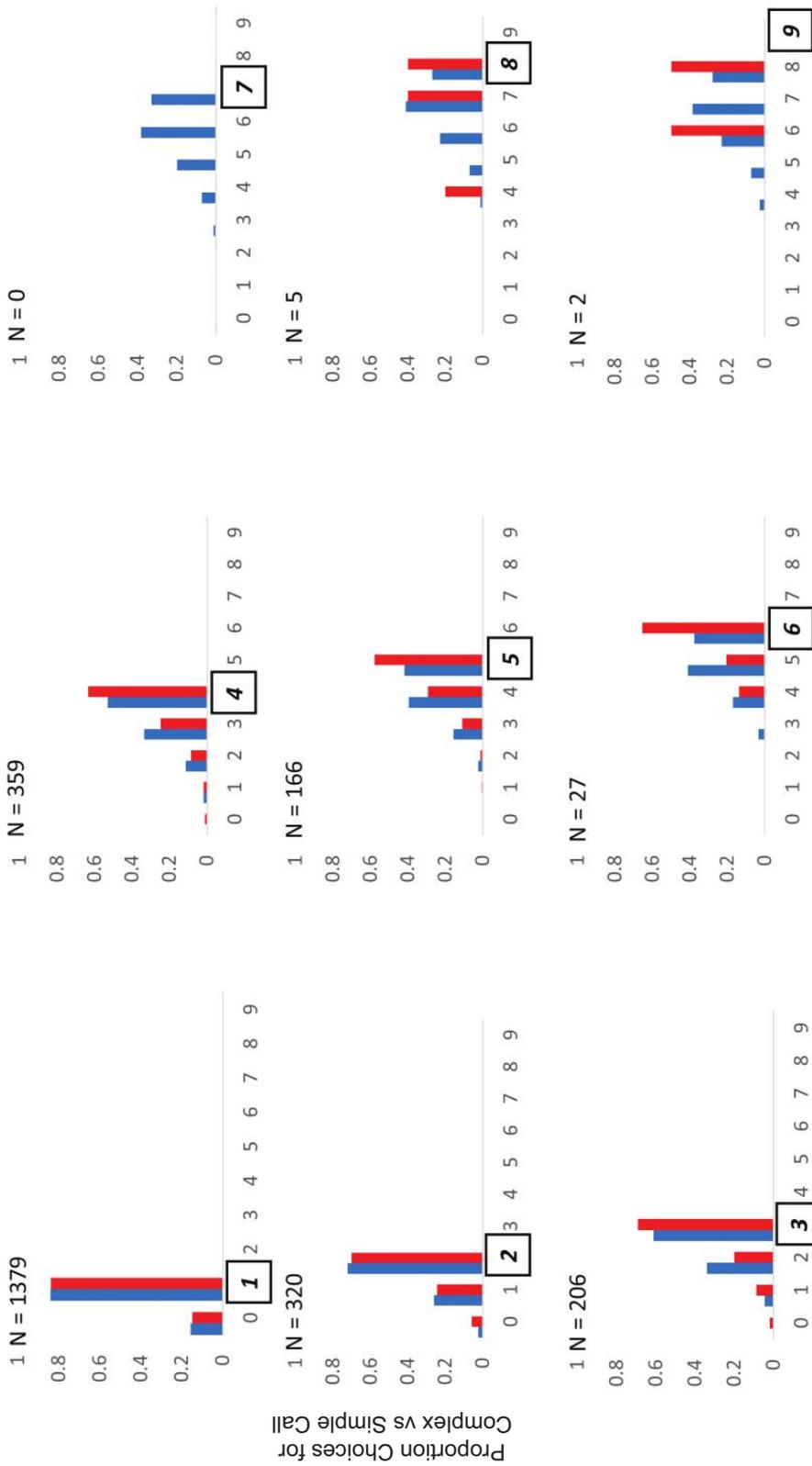
Figure 3: Graphs showing the number of times that females chose the whine-chuck versus the whine when tested between one and nine times (red bars). The numerals highlighted on the X-axes are the numbers of times females in that data set were tested. Females were analyzed only once, each in the data set in which they were tested the maximum number of times. For example, the middle graph on the bottom presents data for females that were tested exactly six times and show the number of tests (zero to six) in which the females preferred the complex call to the simple call. The blue bars show results of simulations of choices of 1,000 "females" indicating the expected distribution of choices if every female had the same strength of preference (0.86).

Table 1: Comparison of models accounting for preference rate variation between individuals and/or years

Model	$\hat{\sigma}^2$	$\hat{\tau}^2$	AIC	ΔAIC
Equation (1)	.788	.109	4,064.7	0
Equation (9)	…	.171	4,100.9	36.18
Equation (10)	1.782	…	4,070.6	5.82
Equation (11)	…	…	4,157.7	92.98

Note: The model column references the equation number defining the model, $\hat{\sigma}^2$ indicates estimated variance Var[F_i] across individuals, and $\hat{\tau}^2$ indicates estimated variance Var[Y_t] across years. The Akaike information criterion (AIC) column gives the AIC value for the indicated model, and the ΔAIC column indicates the difference between the AIC for each model and the AIC for the best-fitting model.

of the same species, Qvarnström et al. (2000) argue that there is adaptive variation in preferences among females within a breeding season, but again there are no data examining inferred preferences across years. One study that examined mating preferences across years was that by Chaine and Lyon (2008), who, like the previous authors, also inferred mate preferences from examining pairing data. They showed that inferred mating preferences for lark buntings vary considerably among male traits and even reversed direction in some cases over just a 5-year period. These studies, combined with the notion that behavior in general and mate preferences in particular are quite variable, reinforce what seems to be a consensus that the strength of mating preferences are not consistent over years (Iwasa and Pomiankowski 1995; Gosden and Svensson 2008; Kasumovic et al. 2008; Cornwallis and Uller 2010).

In this study, we show that there is substantial variation among females tested multiple times in the strength of their preference for complex calls over simple ones. Nevertheless, despite the fact that there is interindividual variation in this preference in túngara frogs, the preference has a constant direction, favoring complex calls over simple calls, and is consistently strong over the 19 consecutive years in which it was measured. There is, however, noticeable variation in the strength of preference among some of the years. These data reject the notion that if there is variation among females in strength of preferences, this necessarily results in a lack of consistency of preferences among years. This clearly is not the case in this data set. The causes of these two types of variation, among females and among years, have yet to be explored.

We do know that there are a number of factors that can influence acoustic mate preferences in these frogs, including light level (Rand et al. 1997), distance/amplitude of the call (Ryan and Rand 1990), perceived predation risk (Bonachea and Ryan 2011), the number of males that are being assessed by females (Lea and Ryan 2015), the degree to which females are gravid (Baugh and Ryan 2009), and circulating hormone levels (Lynch et al. 2006). Among the six studies of these

factors, only those of light levels and call distance/amplitude involve preferences for simple versus complex calls. In addition, other studies of túngara frogs have shown that there was significant variation among females in the degree to which they generalize signals, even though females were not very consistent (Ryan et al. 2003). However, another study showed that the lack of a population preference between certain pairs of calls was not due to females having competing strong preferences that averaged out to no preference but was due to similar lack of preferences among females (Kime et al. 1998). In summary, depending on the task, female túngara frogs are sometimes quite variable in their acoustic mate preferences and other times not at all variable.

There might be an important difference in the female mating preferences inferred for the passerine birds discussed above and the experimentally demonstrated female mating preferences of túngara frogs for the chuck. This difference might highlight how utilitarian and aesthetic female preferences vary. In the study of the passerines cited above, it is argued that females assess male traits that indicate performance in different ecological conditions. The preference for chucks by túngara frogs, as we have argued elsewhere, seems to involve sensory exploitation (Ryan et al. 1990).

In contrast to birds, frogs have two inner ear organs: the amphibian papilla (AP) and the basilar papilla (BP). In most if not all frogs studied to date, there is a good match between the most energetic frequencies in the frog's mating call and the tuning of the inner ear. In some frogs only the AP or only the BP is recruited in communication, while in some others both inner ear organs are involved in communication (Gerhardt and Schwartz 2001; Gerhardt and Huber 2002). The túngara frog is one of the latter; the dominant frequency of the whine matches the most sensitive frequency of the AP, while the dominant frequency of the chuck tends to match that of the BP (Ryan et al. 1990). In other closely related species in the genus *Physalaemus*, most species do not add a chuck-like component to their whine-like mating calls, and the dominant frequencies of their whines tend to match the sensitive frequencies of their AP. Even though these other species do not use the BP in communication, all but one of the eight species examined have a BP tuning that is statistically similar to that of the túngara frog (Wilczynski et al. 2001). These data suggest that the spectral properties of the chuck evolved to match the preexisting tuning of the BP rather than the tuning of the inner ear evolving to match the spectral quality of the chuck. It is thought that this excitation of both inner ear organs by complex calls and the resulting enhanced excitation and functional connectivity in sensory, sensory-motor, and motor areas in the brain (Hoke et al. 2004, 2005, 2007) contribute to the greater female preference for complex calls over simple calls. Although sensory tuning clearly can evolve, we doubt that it is as sensitive to the vagaries of the environment, such as rainfall, that influence male

sexual traits and the preferences that females have for them in some other species.

Another important factor that differs between túngara frogs and songbirds is that frogs in general do not learn their species-specific mating call (Gerhardt and Huber 2002). We know specifically in túngara frogs that males do not learn their calls (Dawson and Ryan 2009), nor do females learn their call preferences (Dawson and Ryan 2012). In songbirds, however, both conspecific songs and the preferences for those songs are learned (Catchpole and Slater 2003), and sexual imprinting is thought to play an important role in speciation in some groups of birds (Grant and Grant 2018). Learned traits are susceptible to influence from changes in the social and physical environment, and change can spread through a population quickly even within a generation. Lack of learning might explain why this particular preference in túngara frogs—the preference for complex calls over simple calls—is not as variable as some other preferences in this frog, as we discussed above. The preference for chucks in túngara frogs is apparently much more stable than mating preferences shown in some other species, especially in passerines, which constitute a sizable portion of sexual selection studies.

In summary, we show that strong and consistent mating call preferences of túngara frogs across years are evident despite significant variation among females. It seems that most females have strong preferences for complex calls, but some are significantly stronger than others. In general, our study reinforces the notion that sexual selection by mate choice has been a potent force generating much of the spectacular biodiversity we see in the animal kingdom. We also humbly suggest that although Alfred Russell Wallace was correct about many things, he was wrong about sexual selection.

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The túngara frog, *Physalaemus pustulosus*. Photo credit: Ryan Taylor.