

EVOLUTIONARY BIOLOGY

Inconsistent sexual signaling degrades optimal mating decisions in animals

Jessie C. Tanner^{1,*†} and Mark A. Bee^{1,2}

Like political stump speeches and product advertisements, animal signals are highly repetitive and function to persuade receivers to adopt behaviors benefiting the signaler. And like potential constituents and consumers, receivers assess signals to inform their behavioral decisions. However, inconsistency in sexual signals is widespread and potentially injects uncertainty into mating decisions. Here, we show that females fail to make optimal mating decisions based on assessments of signal quality due to inconsistency in signal production. Natural levels of inconsistency markedly reduced female preference expression for a nonarbitrary signal of male quality. Inconsistency reshaped preferences even more profoundly than the better-known impediment of ambient noise. To our knowledge, this is the first demonstration of how inconsistent messaging degrades optimal decision-making in animals, with implications for understanding signal evolution.

INTRODUCTION

Animal signals function to persuade or manipulate receivers to adopt behaviors that benefit signalers and thus bear notable similarities with elements of political and marketing campaigns targeted at persuading voters and consumers, respectively (1, 2). Strategies for success in politics and marketing stress consistent messaging (3, 4)—the act of repeating the same information in the same way—as a critical means of influencing the behavioral decisions of voters and consumers. However, despite extensive research on the evolution of animal communication, particularly in the context of mate choice (5, 6), the impacts of consistent messaging on receiver decision-making, and how these impacts determine the strength of sexual selection, remain largely unknown. This is unexpected because animal signaling is inherently repetitive and inconsistent: Most animal signals are performed repeatedly and comprise sequences of repeated elements (7–9) but are repeated with notable inconsistency (10–12). Thus, a fundamental question about animal communication remains: How does inconsistency in signal production affect decision-making by receivers?

Using Cope's gray treefrogs ("gray treefrog"), we tested the hypothesis that inconsistency in signaling degrades the ability of receivers to make optimal decisions based on differences in signal quality. Male gray treefrogs form noisy breeding choruses where they produce energetically expensive "advertisement calls" to attract mates (9, 13). Under optimal conditions (i.e., in quiet using perfectly consistent signals), females prefer longer call durations and faster call rates, two signal features that increase the energetic costs of signaling and potentially function as nonarbitrary signals of male genetic quality (14, 15). In the real world, however, ambient noise impairs females' ability to discriminate between signalers based on individual differences in signaling (16–19). We focused on call rate as a measure of signal quality because it is a common target of sexual selection: Females prefer faster rates of signaling across diverse

taxa, including gray treefrogs (9, 20). Although mean call rate differs reliably among individual gray treefrogs, call rate is also highly variable within individuals over time scales similar to the durations of both single bouts of calling (10, 19) and the assessment windows over which females make mating decisions (21), making call rate an excellent signal feature to model effects of inconsistent signaling on decision-making.

RESULTS AND DISCUSSION

Phonotaxis in response to sequences of synthetic advertisement calls simulating two calling males was used as a behavioral assay in two-alternative choice tests to measure "preference functions" [mating preferences across a range of trait values (22)]. Subjects were given a choice between a "standard stimulus," simulating a male calling at a nominal mean rate near the population mean, and an "alternative stimulus," simulating a second male calling with a nominal mean rate that was 0, 1, or 2 SDs faster or slower than the population mean. We also manipulated the level of inconsistency in both stimuli by varying the magnitude of the within-individual coefficient of variation ($CV_w = SD/\bar{X}$) of the rate at which both simulated males called. We varied CV_w across four nominal levels along a continuum bounded by perfect consistency ($CV_w = 0.000$; Fig. 1A) and by the approximate maximum CV_w within the range of variation in the population ($CV_w = 0.500$; Fig. 1A). These estimates of within-individual variation are based on quantitative analyses of 20 calls recorded within a single bout of calling for each of 50 males (1000 calls total; mean bout duration, 133.5 s; range, 70.4 to 291.3 s) (19). Within a particular choice test, the standard and alternative stimuli were always assigned the same nominal level of inconsistency. Every stimulus sequence used to simulate a calling male was generated by drawing the instantaneous value for call rate (determined as the inverse of the call period) for each consecutive call at random from a normal distribution having the experimentally specified mean call rate and an SD that yielded the experimentally specified CV_w (Fig. 1B) (19). Because the nominal values of call rate and inconsistency were approximated by random draws (Fig. 1B), we determined the realized values of call rate (Fig. 1C) and inconsistency (Fig. 1D) for the standard and alternative stimuli that a subject experienced in a particular choice test as a function of its response latency in that test.

Copyright © 2020
The Authors, some
rights reserved;
exclusive licensee
American Association
for the Advancement
of Science. No claim to
original U.S. Government
Works. Distributed
under a Creative
Commons Attribution
NonCommercial
License 4.0 (CC BY-NC).

¹Department of Ecology, Evolution, and Behavior, University of Minnesota, Saint Paul, MN, USA. ²Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN, USA.

*Corresponding author. Email: jessie.c.tanner@gmail.com

†Present address: Centre for Evolutionary Biology, School of Biological Sciences, University of Western Australia, Crawley, WA, Australia.

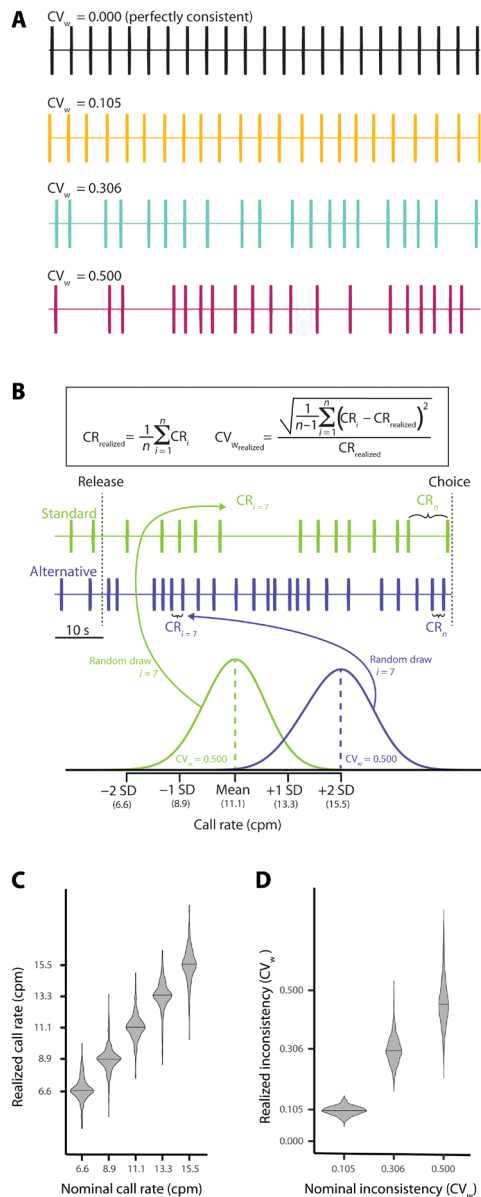


Fig. 1. Acoustic stimuli were generated by random draws from distributions having the experimentally specified nominal call rates and levels of inconsistency. (A) Schematic waveforms showing representative sequences of synthetic calls with the same nominal call rate but different nominal levels of inconsistency (CV_w). (B) Distributions illustrating computations of realized call rate and realized inconsistency based on subject response latency. This example illustrates a choice between a standard stimulus [nominal call rate of 11.1 calls per minute (cpm)] and an alternative with a nominal call rate of 2 SDs faster. Each stimulus comprised a sequence of calls created by randomly drawing the instantaneous call rate of the i th call (CR_i) from a distribution centered on the nominal call rate and having an SD necessary to produce the nominal level of inconsistency (CV_w) for the specified test (here, $CV_w = 0.500$). Also shown are example sequences of calls that a hypothetical subject could have sampled during a two-alternative choice test in the time between being released and making a choice (“response latency”). Only stimulus calls occurring between beginning playback and choice were used to compute realized values for call rate and inconsistency used in analysis and visualization. Equations show how the realized call rate (CR_{realized}) and inconsistency ($CV_{w\text{realized}}$) were computed in each test. (C) Realized call rates (cpm) and (D) levels of inconsistency (CV_w) in relation to their respective nominal levels across all two-alternative choice tests. Horizontal bars in each distribution depict median values.

All statistical analyses are based on realized values of call rate and inconsistency as continuous predictors.

We previously showed that female gray treefrogs discriminate against signal sequences with high levels of inconsistency in call rate, preferring to approach perfectly consistent signals when all other aspects of the two alternatives, including nominal mean call rates, are equal (17). In the present study, although the standard and alternative stimuli used in a particular choice test had the same nominal level of inconsistency, there was potential for small, stochastic differences in the realized level of inconsistency between the two stimuli to affect subject responses. However, we found no evidence that the probability of choosing one stimulus over the other varied as a function of the small difference between their realized levels of inconsistency during trials ($\beta = -0.708$, Wald $X^2 = 0.53$, $P = 0.45$; Fig. 2A). Thus, choice tests were effectively designed to investigate how subjects discriminated between approximately equally variable stimuli on the basis of differences in their mean call rates.

Changes in preference function shape due to signal inconsistency supported the hypothesis that inconsistent signaling degrades optimal decisions. In quiet and when choosing between perfectly consistent signals, females exhibited a linear preference function, strongly favoring faster call rates [Fig. 2, B and F (gray shaded area), and fig. S1]. Females generally continued to prefer faster call rates in quiet across all levels of inconsistency (Fig. 2B). However, they became increasingly less likely to prefer faster call rates as call rates became increasingly inconsistent (Fig. 2B). Moreover, the impact of inconsistency on reducing preferences for faster call rates was greater when call rates were above average. Hence, inconsistency in call rate transformed the preference function in two critical ways expected to affect the strength of selection: It reduced the overall slope of the preference function and it transformed a linear preference into a more curvilinear, threshold-shaped function. The major consequence of these two transformations was the weakening of discrimination against relatively slower call rates and diminished preferences favoring the fastest call rates (Fig. 2B and Table 1).

Because ambient noise is a well-established constraint on communication (23, 24), we compared the relative impact on preference functions of both inconsistent signaling and ambient noise across the natural ranges of both variables (fig. S2). We first evaluated female preferences using perfectly consistent signals presented in biologically realistic levels of ambient noise typical of breeding choruses (17, 18). Noise and its interaction with call rate had significant effects on preference function shape (Table 1). Compared with choices made in quiet (shaded gray area in Fig. 2C), the presence of noise acted, like inconsistency, to reduce the strength of female preferences for faster call rates, an effect that was more pronounced at higher levels of noise. Across the range of biologically relevant values, however, inconsistency had much more profound impacts than noise on reshaping preference functions (cf. Fig. 2, B and C). We also evaluated the joint effects of simultaneous variation in inconsistency and ambient noise (Table 1). Both had significant effects on the shape of preference functions that varied significantly across the range of call rates: Preferences for faster call rates were diminished, and these effects were most pronounced, when call rates were above average (Fig. 2, D to F). Inconsistency and noise both affected preference functions, but their effects were not synergistic.

Receivers might be expected to mitigate the impacts of inconsistent signaling by extending their assessment windows because listening for longer would provide better information about a signal’s “true”

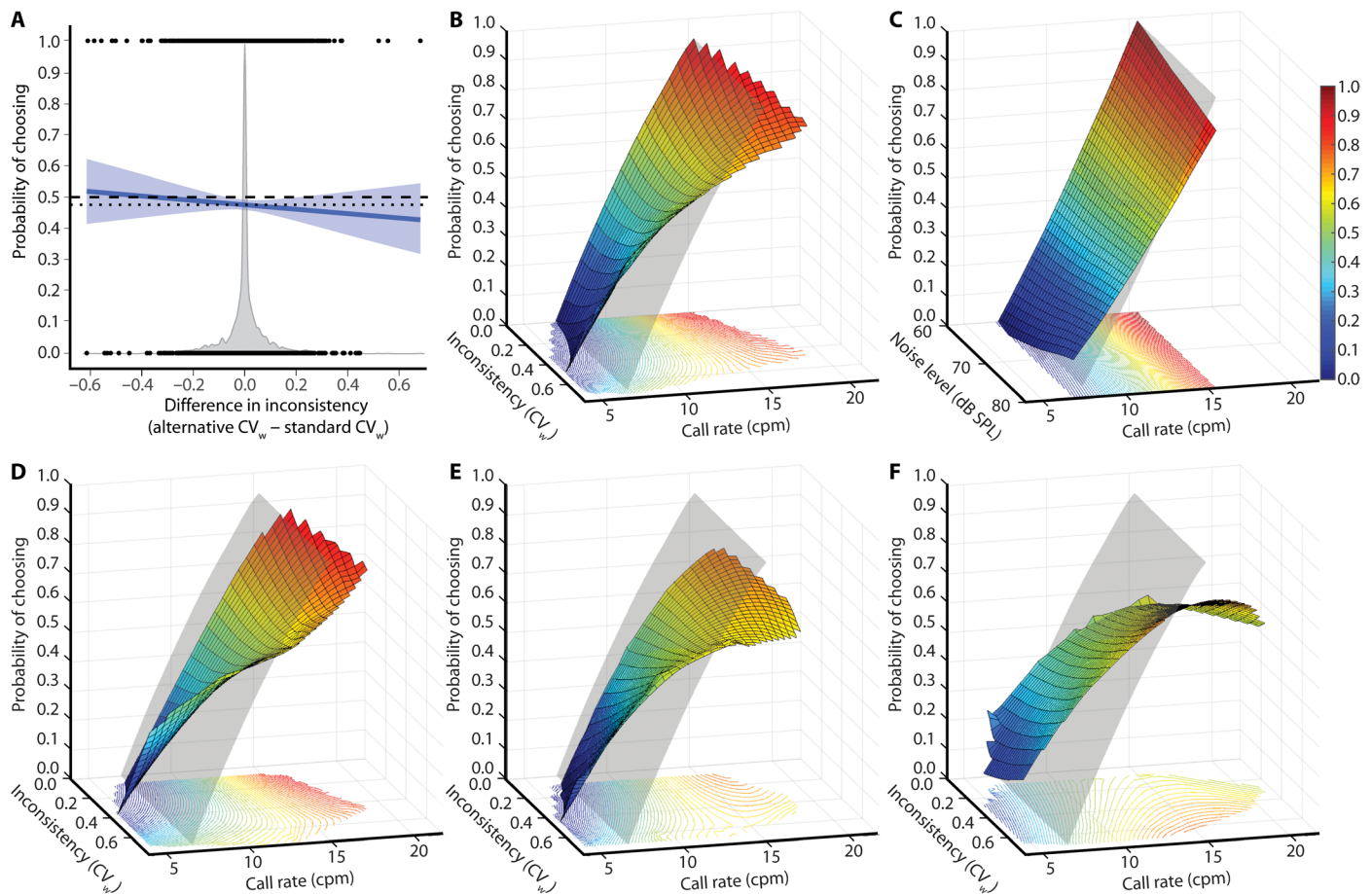


Fig. 2. Inconsistent signaling and ambient noise transformed preference functions by degrading preferences for faster call rates. (A) Stochastic differences in realized inconsistency between standard and alternative stimuli had no impact on responses within choice tests. Points depict the probability of choosing the alternative stimulus (0 or 1) in relation to differences in realized inconsistency between the alternative and standard stimuli during trials, which are depicted by the gray probability density function. Positive x values indicate that the alternative was relatively more inconsistent, while negative x values indicate that the standard was relatively more inconsistent. The dashed line shows the probability of choosing at random (0.500), while the dotted line shows the overall probability with which females chose the alternative call across all experiments (0.475). (B to F) Three-dimensional surfaces illustrate how the linear preference for faster call rates measured under optimal conditions (transparent gray surface) became shallower and more curvilinear, indicating degraded female decision-making. The z axes show the probability of choosing the alternative stimulus, whose mean call rate was $0, \pm 1$ SD, or ± 2 SD different from the mean call rate of the standard stimulus (11.1 cpm). Preferences measured (B) in quiet as a function of call rate and inconsistency; (C) using perfectly consistent signals in ambient noise as a function of call rate and noise level; (D to F) as a function of call rate and inconsistency in ambient noise presented at sound pressure levels (SPLs) of (D) 60 dB, (E) 70 dB, or (F) 80 dB. Surfaces in (B) to (F) were loess smoothed.

quality (Fig. 3A). Consistent with this expectation, the mean (\pm SD) response latency increased by 33.1% (28.5 s) as a function of increasing inconsistency, ranging from 86.0 ± 45.3 s with perfectly consistent signals to 114.5 ± 67.4 s at the highest level of inconsistency when tests were performed in quiet (Fig. 3A and Table 2). Within the time frame over which the vast majority of choices were made (<150 s; Fig. 3A), however, listening longer to more variable signals did not improve the outcome of female decisions (Fig. 3B). Instead, the likelihood of choosing the stimulus with the faster call rate actually declined slightly as a function of increasing latency (Fig. 3B; $\beta = -0.002$, Wald $X^2 = 6.67$, $P = 0.010$). Thus, the small amount of plasticity in assessment windows was insufficient to maintain the preferences that were expressed under optimal conditions (Fig. 2B). A lack of sufficient plasticity may reflect the importance of other biological factors, such as predation risk or limited attention and memory, that constrain how long receivers can afford to assess signals under natural conditions.

Given that receivers do not adequately compensate for signal inconsistency by increasing their assessment times, males producing low-quality signals might benefit from occasionally attempting to subvert female decision-making using short bouts of high-quality signaling. Consistent with this expectation, inconsistency in call rate was negatively related to mean call rate across males' recorded calling in the ponds where female subjects were collected ($F_{1,48} = 19.1$, $P < 0.0001$, $R^2 = 0.29$; Fig. 4A). Males that called at unattractive, low rates on average occasionally produced short sequences of calls at higher rates, whereas males that produced high-quality signals on average did so more consistently (Fig. 4, B and C).

An important finding from this work is the empirical demonstration that inconsistency in sexual signal production potentially affects the evolution of communication systems by altering the strength of sexual selection. Our results show how natural levels of inconsistency in repetitive sexual signaling, especially when combined with the more familiar impediment of ambient noise, significantly

Table 1. Results from GEE models for the effects of inconsistency (in quiet), ambient noise (with perfectly consistent signals), and the joint effects of inconsistency and ambient noise on the probability of choosing the alternative stimulus.

Modeled effects	Parameter	Estimate	SE	Wald χ^2	P value
Inconsistency (in quiet)	Intercept	-0.763	0.044	301.7	<0.001
	Call rate	0.111	0.004	735.4	<0.001
	Inconsistency	1.272	0.210	36.9	<0.001
	Call rate \times inconsistency	-0.114	0.019	36.8	<0.001
Ambient noise (with perfectly consistent signals)	Intercept	-0.711	0.069	106.9	<0.001
	Call rate	0.104	0.006	292.2	<0.001
	Noise	0.199	0.033	35.7	<0.001
	Call rate \times noise	-0.016	0.003	32.1	<0.001
Inconsistency and ambient noise	Intercept	-0.864	0.101	73.5	<0.001
	Call rate	0.112	0.009	170.5	<0.001
	Inconsistency	0.960	0.277	12.0	<0.001
	Noise	0.206	0.045	20.8	<0.001
	Call rate \times inconsistency	-0.069	0.020	11.7	<0.001
	Call rate \times noise	-0.017	0.004	22.4	<0.001
	Inconsistency \times noise	-0.004	0.083	0.0	0.963

reduce the mating advantages of especially attractive males, thereby sheltering less attractive (and potentially lower quality) males from selection. This finding has critical implications for the study of signal evolution. Experiments using perfectly consistent signals almost certainly overestimate the strength of selection, although they are used widely to infer the fitness landscape of signalers (25), make inferences about the limits of sexual signal elaboration (26), and quantify the extent of premating isolation between diverging populations (27, 28). This is likely why measures of selection differentials or mating success in natural settings so often fail to find evidence of the same sexual selection inferred from controlled laboratory experiments (29, 30). It also helps explain why among-individual variation in signals persists in the face of what appears to be strong phenotypic selection (the lek paradox) (20, 31, 32). Future studies of signal evolution must contend with the fact that, in nature, signals are produced with a notable amount of inconsistency (10–12) that degrades optimal decision-making by receivers.

A second key finding is that inconsistent signaling had more profound impacts than noise in diminishing preferences for males producing high-quality sexual signals. This result is important in light of the well-known negative impacts of noise, including anthropogenic noise, on communication systems (23, 24). Considered within the framework of signal detection theory (23), both inconsistency and noise create uncertainty in signal-discrimination tasks, although the underlying perceptual mechanisms likely differ. By observing a sequence of repeated signals, receivers effectively sample from a distribution to estimate a signaler's mean trait value. Inconsistency in signal production increases uncertainty by broadening these distributions. In the absence of sufficient plasticity in assessment windows, as we found here, the inevitable outcome of inconsistent signaling will be degraded decision-making: Females will become more likely to mate with low-quality males (i.e., commit false alarm errors) and less likely to mate with high-quality males (i.e., commit missed detection errors). In gray treefrogs, low-quality

signalers appear to exploit constraints on receiver assessment by engaging in brief episodes, or “sound bites,” of high-quality signaling. Thus, inconsistent messaging against a backdrop of noise and constrained opportunities for assessment may “level the playing field,” allowing low-quality signalers to achieve matings. Given the parallels in signaling between animal communication, politics, and advertising (1, 2), it would be interesting to consider whether some inferior candidates or products associated with inconsistent messaging nevertheless garner broad public appeal due to constraints on more thorough assessment by audiences.

METHODS

Frogs used in this study

Cope's gray treefrog (*Hyla chrysoscelis*) is a widely distributed species found throughout much of eastern North America, where it typically breeds in ponds or wetlands between April and July each year. The species has been the subject of numerous studies of acoustic communication and sexual selection, and its behavior in these contexts is well characterized (33). The present study used as subjects 256 gravid females of the western mitochondrial DNA lineage (34) that were captured in amplexus at night (2200 to 0200) between mid-May and early July in 2015, 2016, and 2017. Gravid female treefrogs captured in amplexus are as discriminating as females captured before making a mating decision (35). Capture locations were located in east-central Minnesota: Carver Park Reserve (44.52490, -93.43031; Carver County), Lake Maria State Park (45.32012, -93.94389; Wright County), Richardson Nature Center (44.84214, -93.37148; Hennepin County), and Crow-Hassan Park Reserve (45.19471, -93.65368; Hennepin County). Pairs were housed in small plastic containers and returned to the laboratory, where they were placed in aged tap water and maintained at approximately 2°C for up to 36 hours to prevent egg deposition. Before testing, pairs were placed in room temperature water and housed in an incubator

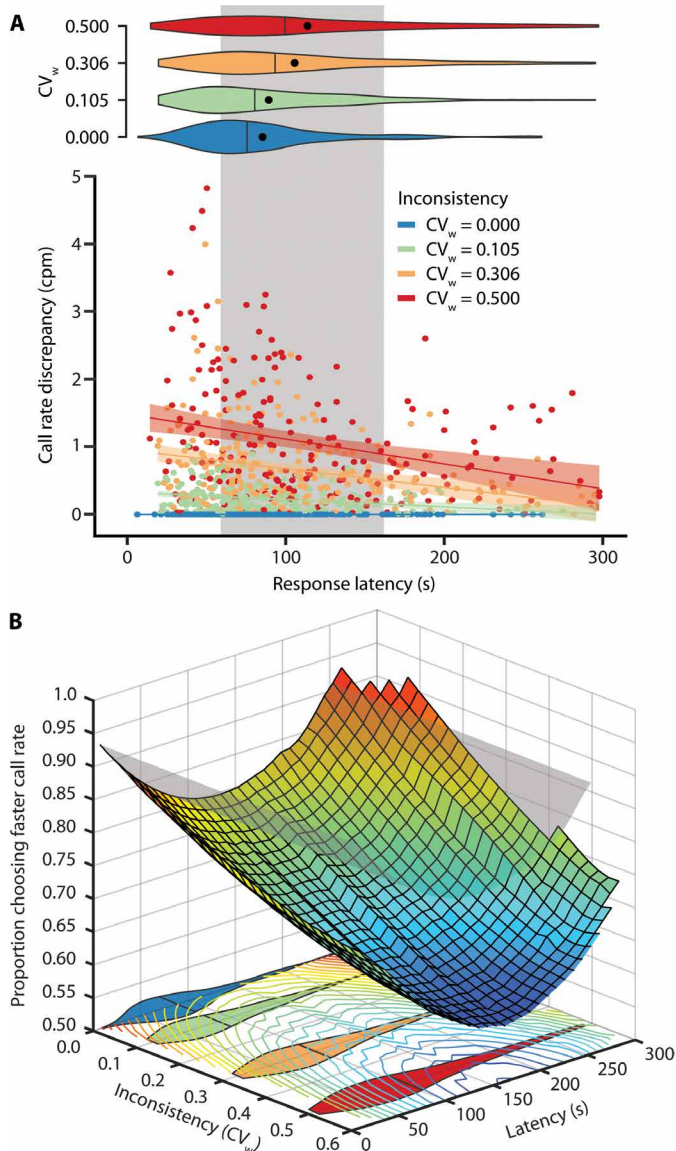


Fig. 3. Increasing response latency improved available information but did not rescue call rate discrimination from degrading with inconsistency. (A) Subjects had longer latencies at higher nominal levels of inconsistency (violin plots: median, black bar; mean, black dot). Mean and median response latencies fell within the 95% confidence intervals around the mean mate assessment time estimated in a previous study (21) (gray shading). Colored points depict the call rate discrepancy (|nominal – realized|) computed as a function of response latency for 935 phonotaxis tests; colored lines and shading show this effect with 95% confidence intervals for each nominal level of inconsistency. Response latency was negatively associated with the discrepancy between nominal and realized call rates, indicating that listening longer before choosing could allow females to gain additional information, leading to better estimates of call rate. (B) Despite improving available information, increasing latency had negative linear ($\beta = -0.002$, Wald $\chi^2 = 6.67$, $P = 0.010$) and significant quadratic ($\beta = 7.32 \times 10^{-6}$, Wald $\chi^2 = 5.43$, $P = 0.020$) effects on the probability of choosing the faster call rate and thus did not “rescue” preference expression. The colored surface shows the proportion of females choosing the faster call rate as functions of inconsistency and response latency. The gray shaded surface shows the proportion choosing the faster call rate when stimuli were perfectly consistent. The difference between the two surfaces illustrates degraded call rate discrimination that was not rescued by adjusting assessment times. Violin plots reproduced from (A). Surfaces in (B) were loess smoothed.

for at least 30 min, until attaining a body temperature of $20^\circ \pm 1^\circ\text{C}$. Temperature control is important in studies of frog communication because both male signal production and female mating preferences are temperature dependent. Following testing, pairs were released at their original capture sites within 3 days. Animal care and all of the experimental procedures described here were carried out in accordance with institutional guidelines and the laws of the United States.

Acoustic recordings and analyses

Analyses of male calling behavior were based on recordings made of calling males in our study population. A total of 20 advertisement calls were recorded for each of 50 actively calling males (1000 calls total) at night (2200 to 0100 hours) during active breeding choruses between May and July of 2006 and 2009. Recordings were made using a Sennheiser ME66 microphone with a K6 power supply (Sennheiser USA, Old Lyme, CT, USA) and a Marantz PMD670 Recorder (D&M Professional, Itasca, IL, USA). The microphone was held by hand or positioned on a tripod such that the recording tip of the microphone was approximately 1 m from the calling male. All recordings were made in the dark, and care was taken to allow the recorded male to adjust to any disturbance presented by the arrival of the human observer. Recordings only commenced after subjects had resumed normal calling behavior following any disturbance.

The durations of calling bouts and the instantaneous call rate (equivalent to the inverse of the call period) for each recorded advertisement call were determined using Raven v1.3 (Cornell Lab of Ornithology, Ithaca, NY, USA). Call rates were temperature corrected to 20°C following the methods of Platz and Forester (36). Coefficients of variation within males were computed as each individual male’s SD in call rate scaled by his own mean call rate across the 20 calls ($CV_w = SD/\bar{X}$). We used the curve estimation function in SPSS version 21 (IBM Corporation, Armonk, NY, USA) to examine the relationship between call rate and inconsistency.

Acoustic stimuli

Synthetic stimuli and ambient noise were generated de novo using custom scripts in MATLAB versions 2015a and 2016a (MathWorks, Natick, MA, USA); all stimuli had a bit-depth of 16 and a sampling rate of 44.1 kHz. Each of the two stimuli used in two-alternative choice tests consisted of a sequence of identical synthetic calls designed to simulate a sexually advertising male. Each stimulus call was modeled after natural calls and had acoustic properties near the previously reported population means (19). The call consisted of 30 pulses (10.2-ms pulse duration, 20.5-ms pulse period, and 50% pulse duty cycle) delivered at a rate of 48.8 pulses/s. Each pulse was constructed by adding two phase-locked sinusoids with frequencies (and relative amplitudes) of 1250 Hz (-11 dB) and 2500 Hz (0 dB). The amplitude envelope of each pulse was shaped to have species-typical onsets and offsets and that of the call was shaped with a 50-ms linear onset. The sound pressure level (SPL; re $20 \mu\text{Pa}$, fast root mean square, C-weighted) of each stimulus in a choice test was calibrated to 85 dB at a distance of 1 m. This sound level approximates that of a natural call (37). Previous studies have shown similar acoustic stimuli to be effective at eliciting phonotaxis behavior from gravid females (17, 19, 33, 38). Across different choice tests, we manipulated the mean rates at which this call was produced and the consistency with which it was produced.

Ambient noise was constructed by filtering white noise to have the average long-term spectrum of a gray treefrog chorus following

Table 2. Results from GEE models for the effects of inconsistency (in quiet) on response latency.

Modeled effect	Parameter	Estimate	SE	Wald χ^2	P value
Inconsistency (in quiet)	Intercept	86.018	8.106	112.6	<0.001
	Call rate	0.022	0.708	0.0	0.975
	Inconsistency	125.475	35.434	12.5	<0.001
	Call rate \times inconsistency	-5.915	2.955	4.0	0.045

established methods (17, 39). We ensured that our results would be broadly applicable to chorus-shaped noise rather than an artifact of a particular realization of a randomly generated stimulus by replicating the noise files. We assigned no more than eight subjects to the same noise replicate. Noise replicates ($n = 38$) were generated at a sampling frequency of 11.025 kHz and then up-sampled to 44.1 kHz. We broadcast ambient noise at C-weighted equivalent continuous SPLs (LCEq) of 60, 70, or 80 dB measured at a distance of 1 m at the subject release site during a choice test.

Experimental design

To characterize the shapes of call rate preference functions, and how they changed in response to the introduction of inconsistency and ambient noise, we measured preference between -2 and $+2$ SDs of the population mean call rate. We used a 4 (choice tests, within subjects) $\times 4$ (ambient noise level, within subjects) $\times 4$ (inconsistency level, between subjects) factorial design in which each female was tested in up to 16 behavioral trials ($n_{\text{subjects}} = 212$; $n_{\text{trials}} = 3,091$). Each subject was tested in four choice tests in which the standard stimulus with a nominal call rate specified as the population mean was presented against alternatives with nominal call rates specified as the population mean ± 1 SD or ± 2 SD. All four choice tests were replicated within subjects at each of four levels of ambient noise (quiet, 60 dB, 70 dB, and 80 dB). Separate groups of females were tested at each of four levels of inconsistency based on measures of within-individual coefficients of variation determined from our acoustic recordings ($CV_w = 0.000, 0.105, 0.306, 0.500$; Fig. 1A). An additional set of 44 individuals was assigned to a control condition, in which females chose between two stimuli that each had a nominal call rate specified as the population mean (i.e., a 0 SD difference). This test was replicated within subjects across the 4×4 factorial combinations of ambient noise and inconsistency ($n_{\text{subjects}} = 44$; $n_{\text{trials}} = 635$). In the control condition, one of the two stimuli was arbitrarily designated as the standard call, and the other was considered to be the alternative. Target levels of inconsistency were chosen because they represented the minimum, mean, and approximate maximum of previously published levels of CV_w for call rate (19). The levels of ambient noise were chosen because they approximate the mean (70 dB SPL) and ± 1 SD (10 dB) of SPLs recorded in gray treefrog choruses in east-central Minnesota (17). Under the “quiet” condition, no noise was broadcast.

To incorporate inconsistency in stimuli (Fig. 1A), we drew the value of the instantaneous call rate (the inverse of the call period) of each consecutive call in the stimulus sequence at random from a normal distribution (Fig. 1B). The SDs of these distributions varied according to the target level of inconsistency, such that we constructed more variable stimuli by drawing from wider distributions. The standard stimulus had instantaneous call rates drawn at random

from distributions centered at 11.1 calls per minute (cpm), the call rate that produces the population mean call effort (call duration \times call rate) when call duration is held constant at the population mean of 30 pulses (Fig. 1B). In each test, the alternative stimulus had instantaneous call rates drawn at random from distributions centered at -2 , -1 , 0 , $+1$, or $+2$ SD away from the nominal call rate of the standard stimulus, which corresponded to call rates of 6.6, 8.9, 11.1, 13.3, or 15.5 cpm, respectively (Fig. 1B). The 0 SD condition served as a control in which females chose between a standard stimulus and an alternative stimulus of nominally equal attractiveness to test for any side bias in our experimental setup. No side bias was detected. Because this study aimed to investigate how natural levels of inconsistency in signals influence female mating decisions, we ensured that synthetic signals contained no supernormal trait values by eliminating randomly drawn values that fell outside the natural range of variation measured in the population (19).

Experimental apparatus and testing protocol

Our test procedures were similar to those published in other studies of communication in gray treefrogs (17, 19, 38, 40–42). Two-alternative choice tests were carried out at $20^\circ \pm 1^\circ\text{C}$ in a custom-built, temperature-controlled, semi-anechoic sound chamber (2.8 m by 2.3 m by 2.1 m, length by width by height; IAC Acoustics, North Aurora, IL, USA). The interior walls and ceiling of the chamber are acoustically insulated and covered in dark gray, perforated metal (IAC Planarchoic panel system). The chamber floor was covered in low-pile, dark gray carpet. Inside the chamber, a circular testing arena (2 m by 0.6 m, diameter by height) was constructed from hardware cloth covered in black fabric. In the center of the arena, an acoustically transparent, circular release cage (9 cm by 2 cm, diameter by height) was placed on the floor. The release cage could be opened by means of a rope and pulley system operated by an observer outside of the chamber. Two Mod 1 Orb speakers (Orb Audio, New York, NY, USA) were used for sound playback and were positioned on the floor of the chamber outside the circular arena wall and positioned 90° apart. Phonotaxis trials were conducted under infrared light (Tracksys Ltd., Nottingham, UK) and observed from outside the chamber using a closed-circuit television system with an infrared-sensitive video camera (Panasonic WV-BP334; Panasonic Corporation of North America, Secaucus, NJ, USA) mounted on the ceiling directly above the release cage. We scored the trials in real time.

We broadcast sounds using Adobe Audition 3.0 (Adobe Systems Inc., San Jose, CA, USA) on a Dell Optiplex 980 PC (Dell Computer Corporation, Round Rock, TX, USA). Audio was output from the computer using a MOTU model 16A 16-channel sound card (MOTU Inc., Cambridge, MA, USA) and amplified using Crown XLS1000 High-Density Power Amplifiers (HARMAN Professional, Northridge,

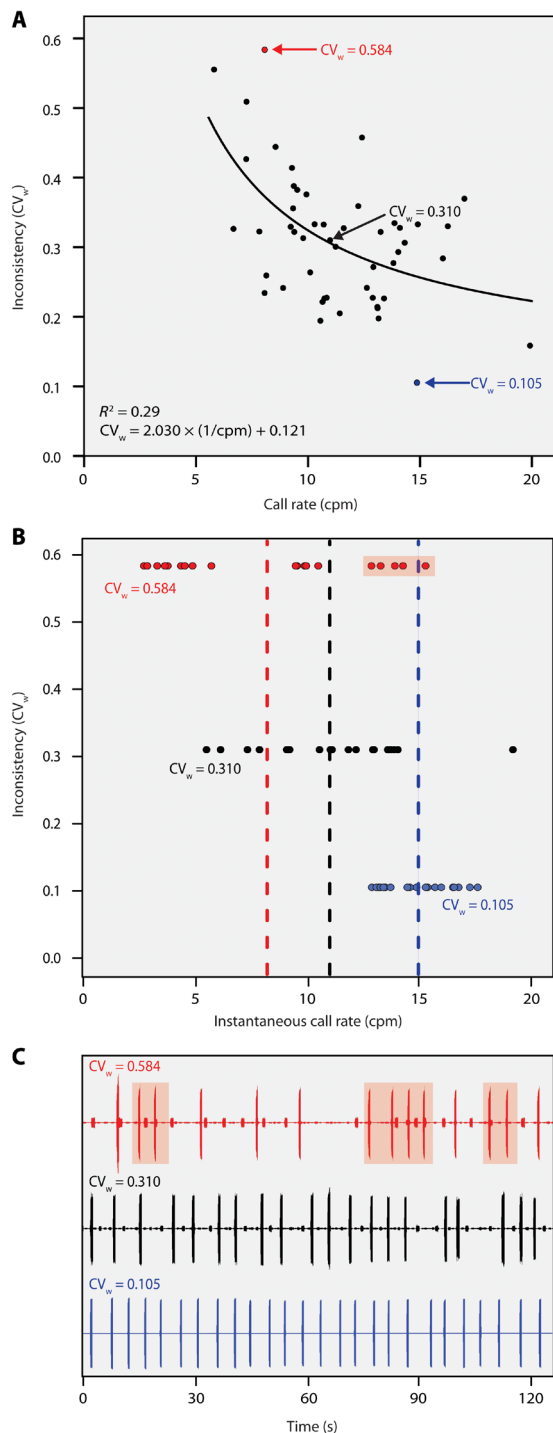


Fig. 4. Inconsistent signalers produced brief sound bites of high-quality signaling in otherwise low-quality bouts. (A) Inconsistency (CV_w) in instantaneous call rate (the inverse of the call period) was inversely related to mean call rate. Points depict mean values measured across 20 calls from each of 50 males ($n = 1000$ calls). Arrows point to data from three males who called with levels of inconsistency representing the population minimum (blue), maximum (red), and approximate mean (black). The call rates (B) and waveforms of 2 min of continuous signaling (C) for the same three males in (A) illustrate how males with low average call rates (red points, lines) nevertheless produced short call sequences at high rates (sound bites, shaded red rectangles), whereas males calling at high average rates (blue points, lines) did so consistently. Vertical dashed lines in (B) depict each male's mean call rate.

CA, USA). Signals and noise were calibrated to their target SPLs using a Type 2250-L sound level meter (Bruël and Kjær, Nærum, Denmark) with a Bruël and Kjær Type 4950 microphone placed at the approximate position of a female's head in the release cage, 1 m from the speaker. Signals and ambient noise were co-located, that is, broadcast from the same speaker. Each of the two speakers played the same ambient noise replicate simultaneously. We calibrated ambient noise to its target SPL while simultaneously broadcasting noise from each speaker, such that each contributed equally to the summed SPL.

To control for presentation order and to prevent side bias, we randomized the trial order, the speaker assignments for the standard and alternative stimuli and whether the standard or alternative stimulus was broadcast first between subjects. At the beginning of each trial, the subject was separated from her mate and placed in the release cage. Before the beginning of the test, females were allowed to acclimate in quiet for 60 s. At the end of the acclimation period, playback began. The ambient noise, when present, began playing first, followed by the signals 30 s later. Standard and alternative stimuli began with an alternating and nonoverlapping temporal arrangement and were subsequently timed according to their respective call rates. Calls continued to be nonoverlapping when call rates were invariant.

Following at least two presentations of each stimulus (Fig. 1B), the lid of the release cage was lifted and the female was allowed to move freely within the arena. We ended trials when one of the following conditions was met: (i) the subject entered a response zone demarcated by a 10-cm radius semicircle in front of a playback speaker, at which point a choice was scored; (ii) the subject made physical contact with the arena wall for the first time in the quadrant opposite the quadrant separating the two playback speakers; (iii) at the end of 3 min, the subject had not left the release cage; and (iv) at the end of 5 min, the subject had not entered a response zone. A previous study demonstrated that females make their mate choice decisions in choruses after assessing males for approximately 1 to 3 min (21). We recorded each female's binary choice (i.e., standard or alternative) and her latency to respond, measured as the time that elapsed between release from the cage and crossing into the response zone. The mean response latency was 92.5 ± 54.3 s. When a choice was not indicated, the subject was given a score of "no response" for that test and given a subsequent opportunity to complete the same test. Subjects that scored no response twice for the same test ($n = 48$ of 256) were not tested further, but their responses from previously completed tests were included in the analysis. All subjects were returned to the incubator for a "time out" of at least 3 min between tests.

Statistical analyses

The nominal mean call rate of each stimulus sequence was the center of the normal distribution from which the values of individual call rates were drawn at random (Fig. 1B). Thus, for both the standard and alternative stimuli, the realized means and coefficients of variation experienced during a trial were variable and dependent on the response latency of the subject. In trials in which subjects took longer to respond, realized means more closely approached the nominal means specified for each experimental treatment because the subjects effectively drew more values from the specified distributions (Fig. 3A). To account for the actual values experienced, we performed a post hoc calculation of the mean call rate and CV_w of the sequence of signals experienced by each subject during each

choice test. We used the response latency from each trial to exclude stimulus calls that the subject did not hear before indicating a choice. Next, we calculated an average CV_w score as the arithmetic mean of the realized standard CV_w and the realized alternative CV_w ; this was performed to reduce the dimensionality of predictors, and because, by design, standard and alternative stimuli always shared the same nominal CV_w in each choice test, such that on average, the difference between the standard and alternative CV_w was very small (mean = -0.00174 ; 95% of values fell in the interval $[-0.223, 0.208]$; range $[-0.612, 0.682]$; see probability density function in Fig. 2A). The realized values for mean call rate (Fig. 1C) and mean CV_w (Fig. 1D) were used to analyze and visualize the data because stochastic deviations from the nominal means and CV_w in the treatment levels would otherwise introduce error into the analysis. Additionally, in the control condition, this approach allowed us to determine whether the subject discriminated between the two arbitrarily labeled alternatives with equal nominal mean call rates on the basis of their realized mean call rates.

We used generalized estimating equation (GEE) models to examine female behavior. GEE is an extension of generalized linear models designed for use with repeated measures of the same individual and compatible with either binary or continuous response variables (logit and gaussian link functions, respectively) (43). All models were fit using exchangeable correlation structures, in which observations within subjects are assumed to be equally correlated. We used Wald statistics with a significance criterion of 0.05 for hypothesis testing. We fit three separate models to examine the effects of inconsistency on decisions made in quiet, the effects of ambient noise on decisions when signals were perfectly consistent, and the joint effects of inconsistency and ambient noise on call rate discrimination, respectively. To determine whether female decisions were driven by differences in inconsistency between signals, we also fit a GEE model examining the effect of the difference in CV_w between trial stimuli on the probability of choosing the alternative stimulus. An additional GEE model examined the effects of inconsistency, call rate, and their two-way interaction on response latency. Last, we fit a separate GEE model to examine the effects of latency, inconsistency, and alternative mean on the probability of choosing the stimulus with the faster rate. We modeled the effect of ambient noise as a continuous variable because the target levels used in our experiment were selected to reflect features of a continuous distribution of chorus noise levels in nature (fig. S2). Separate models that treated the level of ambient noise as a categorical variable yielded qualitatively similar results (see table S1). These statistical models were fit in R version 3.5.0 (44). Three-dimensional plots in Figs. 2 and 3 were drafted in MATLAB version 2018b (MathWorks, Natick, MA, USA) using loess-smoothed data.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/6/20/eaax3957/DC1>

[View/request a protocol for this paper from Bio-protocol.](#)

REFERENCES AND NOTES

- R. Dawkins, J. R. Krebs, *Behavioural Ecology: An Evolutionary Approach*, J. Krebs, N. Davies, Eds. (Blackwell Scientific Publications, ed. 2, 1978), vol. 2, pp. 380–402; www.biosci.utexas.edu/IB/faculty/mueller/384K/animal_signals.pdf.
- J. R. Krebs, R. Dawkins, Animal signals: Mind-reading and manipulation, in *Behavioural Ecology: An Evolutionary Approach*, J. R. Krebs, N. Davies, Eds. (Blackwell Scientific Publications, 1984), pp. 380–402.
- P. Baines, Voter segmentation and candidate positioning, in *Handbook of Political Marketing*, B. Newman, Ed. (Sage, 1999), pp. 403–420.
- T. Duncan, S. Moriarty, *Driving Brand Value: Using Integrated Marketing to Manage Profitable Stakeholder Relationships* (McGraw-Hill, 1997).
- G. Rosenthal, *Mate Choice: The Evolution of Sexual Decision Making from Microbes to Humans* (Princeton Univ. Press, 2017).
- C. Darwin, *The Descent of Man and Selection in Relation to Sex* (J. Murray, 1871).
- A. Kershenbaum, D. T. Blumstein, M. A. Roch, Ç. Akçay, G. Backus, M. A. Bee, K. Bohn, Y. Cao, G. Carter, C. Căsar, M. Coen, S. L. DeRuiter, L. Doyle, S. Edelman, R. Ferrer-i-Cancho, T. M. Freeberg, E. C. Garland, M. Gustison, H. E. Harley, C. Huetz, M. Hughes, J. H. Bruno, A. Ilany, D. Z. Jin, M. Johnson, C. Ju, J. Karnowski, B. Lohr, M. B. Manser, B. McCowan, E. Mercado, P. M. Narins, A. Piel, M. Rice, R. Salmi, K. Sasahara, L. Sayigh, Y. Shiu, C. Taylor, E. E. Vallejo, S. Waller, V. Zamora-Gutierrez, Acoustic sequences in non-human animals: A tutorial review and prospectus. *Biol. Rev. Camb. Philos. Soc.* **91**, 13–52 (2016).
- J. W. Bradbury, S. L. Vehrencamp, *Principles of Animal Communication* (Sinauer Associates, ed. 2, 2011).
- H. C. Gerhardt, F. Huber, *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions* (The University of Chicago Press, 2002).
- H. C. Gerhardt, Female mate choice in treefrogs: Static and dynamic acoustic criteria. *Animal Behav.* **42**, 615–635 (1991).
- K. L. Shaw, D. P. Herlihy, Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proc. Biol. Sci.* **267**, 577–584 (2000).
- P. Coen, J. Clemens, A. J. Weinstein, D. A. Pacheco, Y. Deng, M. Murthy, Dynamic sensory cues shape song structure in *Drosophila*. *Nature* **507**, 233–237 (2014).
- K. D. Wells, *The Ecology and Behavior of Amphibians* (The University of Chicago Press, 2007).
- A. M. Welch, R. D. Semlitsch, H. C. Gerhardt, Call duration as an indicator of genetic quality in male gray tree frogs. *Science* **280**, 1928–1930 (1998).
- G. M. Klump, H. C. Gerhardt, Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. *Nature* **326**, 286–288 (1987).
- N. Lee, J. L. Ward, A. Vézé, C. Micheyl, M. A. Bee, Frogs exploit statistical regularities in noisy acoustic scenes to solve cocktail-party-like problems. *Curr. Biol.* **27**, 743–750 (2017).
- J. C. Tanner, M. A. Bee, Within-individual variation in sexual displays: Signal or noise? *Behav. Ecol.* **30**, 80–91 (2019).
- M. A. Bee, Treefrogs as animal models for research on auditory scene analysis and the cocktail party problem. *Int. J. Psychophysiol.* **95**, 216–237 (2015).
- J. L. Ward, E. K. Love, A. Vézé, N. P. Buerkle, L. R. O'Bryan, M. A. Bee, Multitasking males and multiplicative females: Dynamic signalling and receiver preferences in Cope's grey treefrog. *Animal Behav.* **86**, 231–243 (2013).
- M. J. Ryan, A. Keddy-Hector, Directional patterns of female mate choice and the role of sensory biases. *Am. Nat.* **139**, S4–S35 (1992).
- J. J. Schwartz, K. Huth, T. Hutchin, How long do females really listen? Assessment time for female mate choice in the grey treefrog, *Hyla versicolor*. *Animal Behav.* **68**, 533–540 (2004).
- M. D. Jennions, M. Petrie, Variation in mate choice and mating preferences: A review of causes and consequences. *Biol. Rev. Camb. Philos. Soc.* **72**, 283–327 (1997).
- R. H. Wiley, Signal detection, noise, and the evolution of communication, in *Animal Communication and Noise, Animal Signals and Communication*, H. Brumm, Ed. (Springer-Verlag, 2013), vol. 2, pp. 7–30.
- H. Brumm, H. Slabbekoorn, Acoustic Communication in Noise. *Adv. Study Behav.* **35**, 151–209 (2005).
- R. Brooks, J. Hunt, M. W. Blows, M. J. Smith, L. F. Bussière, M. D. Jennions, Experimental evidence for multivariate stabilizing sexual selection. *Evolution* **59**, 871–880 (2005).
- K. L. Akre, H. E. Farris, A. M. Lea, R. A. Page, M. J. Ryan, Signal perception in frogs and bats and the evolution of mating signals. *Science* **333**, 751–752 (2011).
- K. S. Pfennig, A. M. Rice, Reinforcement generates reproductive isolation between neighbouring conspecific populations of spadefoot toads. *Proc. R. Soc. B* **281**, 20140949 (2014).
- J. Podos, Acoustic discrimination of sympatric morphs in Darwin's finches: A behavioural mechanism for assortative mating? *Philos. Trans. R. Soc. B.* **365**, 1031–1039 (2010).
- B. K. Sullivan, S. H. Hinshaw, Female choice and selection on male calling behaviour in the grey treefrog *Hyla versicolor*. *Animal Behav.* **44**, 733–744 (1992).
- T. W. Friedl, Individual male calling pattern and male mating success in the European treefrog (*Hyla arborea*): Is there evidence for directional or stabilizing selection on male calling behaviour? *Ethology* **112**, 116–126 (2006).
- A. Pomiankowski, A. P. Møller, A resolution of the lek paradox. *Proc. R. Soc. Lond. B.* **260**, 21–29 (1995).
- J. G. Kingsolver, H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gilbert, P. Beerli, The strength of phenotypic selection in natural populations. *Am. Nat.* **157**, 245–261 (2001).

33. H. C. Gerhardt, Acoustic communication in two groups of closely related treefrogs. *Adv. Study Behav.* **30**, 99–166 (2001).
34. M. B. Ptacek, H. C. Gerhardt, R. D. Sage, Speciation by polyploidy in treefrogs: Multiple origins of the tetraploid, *Hyla versicolor*. *Evolution* **48**, 898–908 (1994).
35. C. G. Murphy, C. H. Gerhardt, Evaluating the design of mate-choice experiments: The effect of amplexus on mate choice by female barking treefrogs, *Hyla gratiosa*. *Animal Behav.* **51**, 881–890 (1996).
36. J. E. Platz, D. C. Forester, Geographic variation in mating call among the four subspecies of the chorus frog: *Pseudacris triseriata* (Wied). *Copeia* **1988**, 1062–1066 (1988).
37. H. C. Gerhardt, Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. *J. Comp. Physiol.* **102**, 1–12 (1975).
38. J. C. Tanner, J. L. Ward, R. G. Shaw, M. A. Bee, Multivariate phenotypic selection on a complex sexual signal. *Evolution* **71**, 1742–1754 (2017).
39. A. Vélez, M. A. Bee, Dip listening and the cocktail party problem in grey treefrogs: Signal recognition in temporally fluctuating noise. *Anim. Behav.* **82**, 1319–1327 (2011).
40. M. A. Bee, E. M. Swanson, Auditory masking of anuran advertisement calls by road traffic noise. *Anim. Behav.* **74**, 1765–1776 (2007).
41. M. A. Bee, Parallel female preferences for call duration in a diploid ancestor of an allotetraploid treefrog. *Anim. Behav.* **76**, 845–853 (2008).
42. K. M. Schrode, J. L. Ward, A. Vélez, M. A. Bee, Female preferences for spectral call properties in the western genetic lineage of Cope's gray treefrog (*Hyla chrysoscelis*). *Behav. Ecol. Sociobiol.* **66**, 1595–1606 (2012).
43. J. Hardin, J. Hilbe, *Generalized Estimating Equations* (Chapman & Hall, ed. 2, 2012).
44. R Core Team, *R: A language and environment for statistical computing* (R Foundation for Statistical Computing, 2018).

Acknowledgments: We thank all members of the Bee lab 2015–2017 for assistance collecting treefrogs and running trials; S. Jørgensen, N. Lee, and C. Donohue for coding advice; A. Vélez, K. Schrode, and J. Ward for help with call recordings and analyses; H. Brumm, H. C. Gerhardt, G. Klump, and J. Schulz for helpful discussions about this work; M. Zuk, R. G. Shaw, H. C. Gerhardt, A. T. Baugh, and two anonymous referees for feedback on an earlier draft of this manuscript; and the Three Rivers Park District and Minnesota State Parks for access to field sites. **Funding:** This work was supported by the National Science Foundation Graduate Research Fellowship under grant no. 00039202, a Ford Foundation Pre-Doctoral Fellowship, an Animal Behavior Society Student Research Grant, and a Joyce Davenport Fellowship in Natural History through the Bell Museum of Natural History to J.C.T. and by a National Science Foundation Grant (IOS-1452831) to M.A.B. **Author contributions:** J.C.T. wrote the software; collected, curated, and analyzed the data; and wrote the original draft. Both authors designed the study, secured funding, supervised the project, made the figures, and edited the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper, the Supplementary Materials, and/or the University of Minnesota repository under accession number <http://hdl.handle.net/11299/212011>. Additional data related to this paper may be requested from the authors.

Submitted 19 March 2019

Accepted 3 March 2020

Published 15 May 2020

10.1126/sciadv.aax3957

Citation: J. C. Tanner, M. A. Bee, Inconsistent sexual signaling degrades optimal mating decisions in animals. *Sci. Adv.* **6**, eaax3957 (2020).