Female discrimination thresholds frequently exceed local male display variation: implications for mate choice dynamics and sexual selection

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Abstract

Among the factors that can influence female mate choice decisions is the degree to which females differentiate among similar displays: as differences decrease, females are expected to eventually stop discriminating. This discrimination threshold, in conjunction with the magnitude of male trait variation females regularly encounter while making mate choice decisions, may have important consequences for sexual selection. If local display variation is above the discrimination threshold, female preferences should translate into higher mating success for the more attractive male. But if display variation is frequently below the threshold, the resulting increased pattern of random mating may obscure the existence of female mate choice. I investigated the interplay between female discrimination and male display variation in green treefrogs (*Hyla cinerea*) and found that call trait differences between nearest neighbour males were frequently smaller than what females are expected to discriminate. This finding has two important consequences for our understanding of sexual selection in the wild: first, low display variation should weaken the strength of selection on male display traits, but the direction of selection should mirror the one predicted from females choice trials. Second, caution is needed when interpreting data on realized mating success in the wild: a pattern of random mating with respect to male display traits does not always mean that female preferences are weak or that conditions are too challenging for females to express their preferences. Rather, insufficient display variation can generate the same pattern.

Introduction

A central question in the study of sexual selection deals with the impact of mate choice in the wild (Hoekstra *et al.*, 2001; Kingsolver *et al.*, 2001). The first step in assessing the impact of mate choice is to describe female mate preferences, which, when compared to the distribution of male traits in the population, generate hypothesis about the direction and strength of selection (e.g. Ritchie, 1996; Rodríguez *et al.*, 2006). Whether and how females express mate preferences, however, is influenced by a range of additional factors (Jennions & Petrie, 1997; Cotton *et al.*, 2006). For example, mate

choice decisions may be influenced by how females perceive and compare displays, in particular the degree to which females differentiate among similar displays (Ryan & Rand, 2003; Phelps *et al.*, 2006; Akre *et al.*, 2011). Rather than discriminating among displays at an infinitely fine scale, females are expected to eventually stop discriminating as differences decrease. The level of difference between two displays at which a female no longer discriminates between them (i.e. their discrimination thresholds) may result from differences being too small to be perceived by the females, or from differences being too small to warrant a differential response from the female (Nelson & Marler, 1990; Ryan & Rand, 2003). Irrespective of the underlying cognitive and selective phenomena, the degree of female trait discrimination should affect patterns of male mating success, and thus, sexual selection. Males with more attractive displays should have higher mating success if

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trait variation is above the discrimination threshold. But if trait variation is below the discrimination threshold, females are not expected to always choose the more attractive male, which may result in a pattern of apparently random mating success. Consequently, the discrimination threshold is an important feature to take into account when examining sexual selection in the wild.

Of complementary importance to discrimination thresholds in females is the amount and spatial and temporal distribution of the male display variation that females will encounter. Unless a female samples every male in the population before making a mate choice decision, which seems an unreasonable scenario, the population-wide trait variation may not be the most biologically relevant measure. Rather, because attractiveness is often not absolute, but a function of the other males with whom the focal male is being compared, the average variation found in the smaller groups of males that are actually sampled by females may be the more biologically relevant range influencing mate choice (Bateson & Healy, 2005; Cotton *et al.*, 2006). If local display variation is insufficient (i.e. below discrimination threshold), females are not expected to discriminate among males. Consequently, to examine the dynamics of mate choice in the wild, it is important to assess the display variation that is most likely to be sampled by females, and the factors that influence this variation.

Display variation may be affected by a range of factors. Overall variation may be reduced by prolonged and/or strong selection by female choice (Andersson, 1994; Reinhold, 2011), whereas local display variation may be influenced by male behaviour or environmental effects. If, for example, males position themselves close to neighbours with similar trait values, variation at the local scale should diminish. Even in the absence of assortative positioning, display variation may be reduced by behavioural adjustments. Males frequently show socially mediated plasticity in display behaviour (i.e. Schwartz *et al.*, 2002), and adjustments of displays to close-by rivals can reduce variation within local groups. Finally, environmental effects such as temperature can influence local display variation. For example, call traits of ectothermic animals like anurans and insects frequently show temperature dependence (Gerhardt & Huber, 2002), and local call variation may be reduced simply because neighbouring males are bound to share a more similar environment than more distant ones.

Anuran amphibians offer a great opportunity to study the importance of male display variation and female discrimination thresholds on mating decisions. Mate choice in anurans is mediated largely via acoustic signals, and detailed information on female preferences is available for many call traits (Ryan, 2001; Gerhardt & Huber, 2002). Less is known about the mate sampling rules of female anurans, but a series of detailed studies of mate assessment behaviour in barking treefrogs (*Hyla gratiosa*) suggests that females sample several males simultaneously from a distance and incorporate into their decisions both the characteristics of male calls and the distance to potential mates (or correlates of distance such as call amplitude). Females frequently approach and mate with the males that are closest to them, and bypass close males only when the additional distance they have to travel to reach a more attractive male is relatively small (Murphy & Gerhardt, 2002; Murphy, 2012). Similar behaviour, where females do not move through the chorus to sample potential mates sequentially, but rather assess only a few close-by males before making their mating decision has been observed in several other species (Martof & Thompson, 1958; Wells & Schwartz, 1984; Grafe, 1997; Gerhardt *et al.*, 1987; Morris, 1989; Murphy & Gerhardt, 2002; Friedl & Klump, 2005; but see Ryan, 1985; Robertson, 1986). Importantly, the observation that proximity appears to be very important in female mate choice decisions highlights the need to focus on local display variation when studying sexual selection in the wild.

Here I study green treefrogs (*Hyla cinerea*) to evaluate (i) the female discrimination threshold, (ii) the magnitude of male display variation that is available for females to choose from when making mate choice decisions, and (iii) how this may affect patterns of mate choice, sexual selection and signal evolution. To evaluate the minimum trait difference that females discriminate, I conducted playback choice trials focusing on call traits that are known to affect female choice in this species (Gerhardt, 1974, 1978, 1987; Höbel & Gerhardt, 2003). I also sampled call differences between nearest neighbour males in the field, thus obtaining an estimate of the magnitude of call differences females are likely to encounter when making mating decisions. To evaluate whether male behaviour such as assortative positioning or behavioural call adjustments affected local display variation, I also looked for patterns of correlation between traits of neighbouring males. I chose pairs of nearest neighbour males as the unit of comparison because in acoustically communicating organisms, female choice is heavily influenced by the distance to potential mates (mediated via signal amplitude), manifest in preferences for closer, that is louder calls (Gerhardt & Huber, 2002). Moreover, preferences for other call traits (i.e. call duration, frequency) can be abolished or reversed by decreasing the amplitude of the more preferred call (reviewed in Gerhardt & Huber, 2002). Females usually prefer the closer of two signals when the source amplitudes are equal (Gerhardt & Huber, 2002; Murphy & Gerhardt, 2002). Consequently, females should perceive males that are each other's nearest neighbours at higher and more similar amplitude than other, farther males, making nearest neighbours – and the magnitude of their call trait

differences – the most likely target of female assessment. Finally, I assessed how often call differences of nearest neighbour males were above the female discrimination threshold, thus obtaining an estimate of how frequently one might expect to find the pattern of male mating success that is predicted from female preference trials. Showing that available mates are frequently too similar to be discriminated would suggest that caution is needed when interpreting observations of realized male mating success not following a preference-trial predicted pattern of male attractiveness. Rather than indicating a lack of mate choice because preferences cannot be expressed due to challenging conditions, it may actually point towards a lack of sufficient variation among potential mates, resulting in very different interpretations for sexual selection and signal evolution. The former would suggest that there is no sexual selection acting on male traits. By contrast, the latter would suggest that selection still operates in the direction that is predicted by female preference trials, but that the strength of selection is reduced because it can only act on the fraction of the male population that is in nearest neighbour associations that allows females to differentiate between them.

Materials and methods

Study site and study species

Study site
The study was conducted in June 2005 at Richland Creek Wildlife Management Area, Freestone Co., Texas. Calls were recorded and analysed (see below) within the first four days of the study. Data on call trait variation (average, range) then served as the basis for generating stimuli for female choice trials, which were conducted over the period of about one week thereafter. Animal use was approved by the Animal Care and Use Committee of the University of Missouri, Columbia (protocol # 1019).

Male call and calling behaviour
Green treefrogs, *Hyla cinerea*, are common inhabitant of lakes, ponds and swamps in the south-eastern United States (Conant & Collins, 1998). The advertisement call consists of a single pulse of about 150 ms duration and is repeated 1–2 times per second. Calls are composed of several harmonics, with emphasis around 900 Hz (also the dominant frequency), 2700 and 3000 Hz.

Males prefer elevated perches, but will call from the ground along the shore if elevated perches are missing (Höbel & Gerhardt, 2003). Distances between nearest calling neighbours are highly variable, and often a function of the local availability of perch sites (Fellers, 1979; Mitchell & Miller, 1991; G. Höbel, pers. obs.). No published data on spatial distribution patterns of male *H. cinerea* exists, but in three undisturbed study sites (i.e. natural ponds and marshes) males called in pairs and rarely in trios; average distances between nearest neighbours at these sites were 3.1, 4.3 and 4.6 m; distances to other nearest neighbour pairs were often 10 m or more (this study, Höbel, 2015).

Female call preferences and mate sampling behaviour
Hyla cinerea females prefer longer calls and shorter call periods (i.e. more frequently repeated calls) (Gerhardt, 1987; G. Höbel, unpublished), and females from this particular study population also prefer lower frequency calls (Höbel & Gerhardt, 2003).

Although published information on mate sampling behaviour in *Hyla cinerea* is not available, it has been studied in detail in its sister species *Hyla gratiosa*, and results from these studies may be pertinent for *H. cinerea* as well. During playback experiments, female *H. gratiosa* may evaluate up to four equally spaced speakers, but they bypass a closer speaker only when the additional distance to a more distant one playing a more attractive call is relatively small (Murphy, 2012). Further, if mate-searching female *H. gratiosa* are followed in the chorus, 95% mate with the closest or second closest male (Murphy & Gerhardt, 2002). Thus, although females may be capable of sampling several males simultaneously, the effect of distance on female mate choice decisions probably limits the choice to the two closest alternatives.

Female discrimination thresholds

I conducted two-choice playback trials to test the minimum trait difference necessary to result in discrimination. Prior experiments had established that females preferred lower call frequency, longer call duration and shorter call periods (Gerhardt, 1987; Höbel & Gerhardt, 2003; G. Höbel, unpublished). This allowed for an *a priori* designation of call alternatives as attractive or unattractive, such that a lack of preference for the attractive alternative could be used as an assay indicating that the presented magnitude of difference between alternatives was below the level at which females discriminate. To distinguish between the more attractive alternative, and the alternative that the female actually chose in the current trials, I adopt the following terminology: the lower, longer or faster alternative presented in each two-choice trial is the 'attractive alternative', whereas the alternative that is approached by the females in each choice trial is the 'chosen alternative'.

Playback system
I tested females in a portable playback arena set up in the field. The arena was 2 m long and 1 m wide. The floor was a plywood board, the sides 50-cm-high wood frames screened with lightweight black cloth. The speakers (RadioShack Optimus XTS-40, RadioShack Corporation, Fort Worth, TX, USA) were placed 2 m

apart, facing each other along the central long axis and just outside the arena. Sound files were broadcast from a laptop at a sampling rate of 44.1 kHz, using CoolEdit96 software (Syntrillium Software Co., Phoenix, AZ, USA). The sound pressure level (SPL in decibels [dB] re 20 μPa, fast root-mean-square [RMS]) of the stimuli was equalized to 85 dB using a Lutron SL-4001 sound level metre.

Synthetic stimuli were generated from 16-bit digital files created by custom-designed software (courtesy of J. Schwartz). Stimuli varied in frequency, duration and call period (see Table 1), but always had a rise time of 25 ms (inverse exponential) and a fall time of 50 ms (inverse exponential).

I obtained females by collecting pairs in amplexus around the peak of male calling activity (21:00–23:00 hours). This assured that females were sexually responsive, and that they had not yet laid eggs, after which they become unresponsive to playback stimuli. All females were tested only once with a given pair of alternatives, and all females responded in the trials they were tested in. I made all behavioural observations under dim red light and released all females at the site of capture within one day of being tested. I did not mark frogs before releasing them. Most female *Hyla cinerea* mate only once, and the ones that do mate multiple times take about 2 week between successive clutches (Perrill & Daniel, 1983). As all females tested in this study were collected over the course of one week, I am confident that I did not retest females.

For testing, I placed females individually in a small round wire cage (10 cm diameter) midway between the loudspeakers. Once the alternative stimuli had been played back for five repetitions, I remotely removed the lid of the release box by pulling a string so that the female could move freely. I scored a positive response once a female touched the cloth in front of a speaker, and noted whether this chosen stimulus was the attractive alternative.

Experimental design

Frog calls are multivariate signals, combining a variety of acoustic traits (e.g. Gerhardt & Brooks, 2009). However, as I was interested in specific call traits that are known to affect female choice in this species (Gerhardt, 1974, 1978, 1987; Höbel & Gerhardt, 2003), I opted to conduct a series of univariate trials for this study.

I conducted three blocks of playback trials, in which I tested discrimination for dominant frequency, call duration and call period, respectively. Within each block, I set the difference between the alternatives of the trait under investigation (say, dominant frequency) to 5%, 10%, 15%, 20% and 30%, while leaving the other two traits (say, call duration and call period) at the mean values found in the study population (see Fig. 1; Table 1). Note that since the tested alternatives bracket the mean, there is no 'standard' against which other alternatives are tested, but rather two alternatives that vary in the magnitude of difference between them. Sample size ranged from $n = 12$–23 tested females per choice trial.

Statistical analysis

I report the proportions of females that chose the attractive alternative (i.e. lower frequency, longer duration, shorter period). I also provide P-values from binomial tests; I used one-tailed tests because I tested the directional prediction that, as long as females can distinguish between alternatives, they will choose the more attractive one.

Male display variation

Call recording and analysis

I used a Sony WM-D6C tape recorder (Sony Corporation, Tokyo, Japan) and a Sennheiser K6+ME66 microphone (Sennheiser Electronic Corporation, Wedemark, Germany) to record the calls of 35 pairs of nearest neighbour males. I defined nearest neighbours as the two frogs with the shortest intermale distance that did not exceed 5 m. Nineteen males were part of more than one nearest neighbour association, such that I recorded a total of 51 different males.

I digitized (44.1 kHz sampling rate) and analysed the recordings using CoolEdit96 (Syntrillium Software Co.). I measured the dominant frequency of the call, the call duration and the call period (time from onset of one call to onset of the following call), and calculated mean values based on the analysis of 10 calls per male.

In this study, I focus on variation among traits in the magnitude of the difference between nearest neighbour males, relating them to female preferences, and comparing this relationship between traits. To facilitate direct comparisons between call traits, I standardized

	Dominant frequency (Hz)	Call duration (ms)	Call period (ms)
Mean (Range)	860 (791–976)	126 (98–155)	488 (388–608)
5% difference	840 vs. 882	123 vs. 129	476 vs. 500
10% difference	817 vs. 903	120 vs. 132	464 vs. 512
15% difference	796 vs. 925	117 vs. 135	451 vs. 525
20% difference	774 vs. 946	113 vs. 139	439 vs. 537
30% difference	731 vs. 989	107 vs. 145	415 vs. 561

Table 1 Call trait values in the study population (top rows) and stimulus values used in female choice trials.
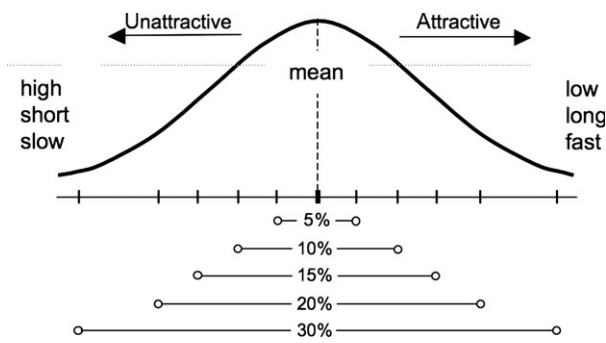
Fig. 1 Schematic description of the experimental design testing female discrimination thresholds. The idealized distribution curve represents variation in male call traits in the population. Each connected pair of circles represents a playback tests (five per call trait). Note that tested alternatives bracket the mean trait value found in the population in increasing degrees of magnitude from 5% difference to 30% difference. See Table 1 for actual trait values of each tested alternative.

variation values by dividing them by the respective population means (Mean$_{Pop}$). Scaling by the mean allows me to generate a dimensionless measure that captures this variation among traits. The alternative (scaling by the SD) would obscure this variation among traits.

Population-wide display variation
To assess the maximum differences of male displays in the population, I calculated the maximum difference for each call trait:

$$\text{Diff}_{Pop} = 100 \times ((\text{Max}_{Pop} - \text{Min}_{Pop})/\text{Mean}_{Pop}).$$

where Max$_{Pop}$ is the maximum, and Min$_{Pop}$ is the minimum trait value in the population for each call trait (frequency, duration and period, respectively); Mean$_{Pop}$ is the mean for each trait in the population.

Within-pair comparisons
To assess average trait variation, I calculated the magnitude of between-male call trait differences of each pair of nearest neighbours males:

$$\text{Diff}_{NN} = 100 \times (\text{ABS}(\text{Mean}_{Male1} - \text{Mean}_{Male2})/\text{Mean}_{Pop}).$$

From this, I calculated the average magnitude of trait differences between nearest neighbour males (mean Diff$_{NN}$), as well as the maximum trait difference observed between nearest neighbours (max Diff$_{NN}$). Note that I used Mean$_{Pop}$ as the denominator when calculating differences between nearest neighbours (i.e. I explore differences between nearest neighbour males with reference to the population mean).

Average trait variation, that is the amount of variation females are likely to sample when making mate choice decisions in the field, may be a function of pop-

ulation-wide trait variation, but it may also be influenced by (i) the composition and behaviour of nearest neighbour pairs, or (ii) the shared environment of nearest neighbour pairs. For example, average variation could be maximized if males with long calls settle next to males with short calls. On the other hand, average variation could be minimized if males with long calls settle next to other males with long calls, or if having a male with long calls settling nearby induces the neighbour to produce longer calls himself. To assess these possibilities, I calculated correlations between call traits of males in each pair. Further, as nearest neighbour males are likely to be at similar temperatures, call similarities between them may arise from independent correlation with temperature. To assess this possibility, I calculated correlations between call traits and temperature. I used JMP 8.0.2 (SAS Institute, Cary, NC, USA) to calculate correlations.

Among-trait comparisons
To assess whether the within-pair trait difference is associated with the amount of variation of a trait in the population, I calculated correlations between Diff$_{Pop}$ and mean as well as max Diff$_{NN}$ for each of the three investigated call traits.

**Estimating mate choice among nearest neighbour males**

I estimated the expected pattern of mate choice (i.e. preference-trial predicted mating success or random mating) in the study population by tallying how frequently within-pair call trait differences were above the threshold for female discrimination. If within-pair difference was above the respective thresholds, I scored the male with the more attractive call (lower frequency, longer call, shorter call period) as having higher mating success. If differences were below the threshold, I scored them as having equal probability of mating. I did so separately for each call trait.

I used two cut-off thresholds obtained from the female choice trials: (i) the trait difference at which > 50% of females discriminate in favour of the more attractive trait value, and (ii) the trait difference at which > 70% of females discriminate in favour of the more attractive trait value. Note that at the sample size in this study ($n = 12–23$), the 70% criterion is equivalent to the trait difference at which a binomial test would be statistically significant at $\alpha = 0.05$.

Results

Female discrimination thresholds

Using the '> 50% of females discriminate in favour of more attractive trait value' criterion, the trait difference required for discrimination of dominant
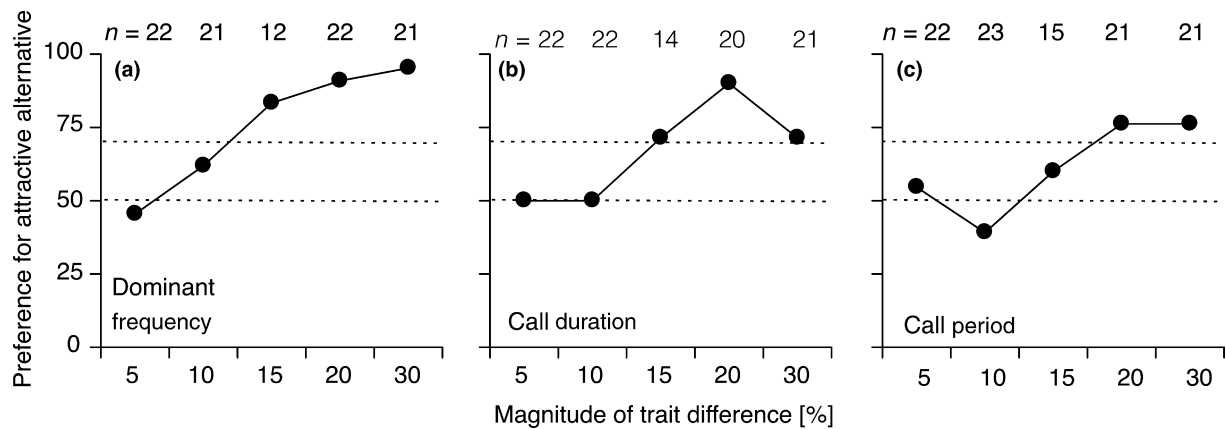
Fig. 2 Results of trials testing female discrimination thresholds. Dashed horizontal lines indicate the two preference criteria used to determine discrimination thresholds (> 50% and > 70% of females discriminating in favour of the more attractive alternative, respectively). Symbols indicate the proportion of females choosing the more attractive alternative [(a) lower frequency, (b) longer duration, (c) shorter period], as a function of the magnitude of difference between the presented alternatives.

frequency is 10%, and that for call duration or call period is 15% (Fig. 2). Using the '> 70% of females discriminate' criterion (equivalent to significance at $\alpha = 0.05$ in a binomial test), the trait difference required for discrimination of dominant frequency or call duration is 15%, and that for call period is 20%, respectively (Fig. 2).

Male display variation

Correlations between call traits
Call frequency was not correlated with other call traits. Call duration was significantly correlated with call period, and both call duration and period were also significantly correlated with temperature (Table 2).

Population-wide display variation
Compared across the entire sample ($n = 51$ males), the maximum difference in male displays (Diff_{Pop}) was 21.4% for dominant frequency, 44.5% for call duration and 44.9% for call period.

Within-pair comparisons
The maximum difference in male displays between two males in a nearest neighbour pair (max Diff_{NN}) was 16% for dominant frequency, 26% for call duration

and 29% for call period. Average differences between nearest neighbours, however, were much smaller: the average within-pair difference (mean Diff_{NN}) was 6.2% for dominant frequency, 11.3% for call duration and 7.9% for call period (Fig. 3).

Dominant frequency was not correlated between neighbouring males (Fig. 4a), suggesting that the low average within-pair difference (6.2%) was not the result of males with similar call frequency settling next to each other. However, both call duration (Fig. 4b) and call period (Fig. 4c) were significantly positively correlated. This correlation may arise from positive assortative positioning of males that have similar calls, from neighbouring males adjusting to each other's calling behaviour, or from independent correlation of call duration and period of males that call at similar temperature.

Among-trait comparisons
Traits that showed higher population-wide variation (i.e. call duration and period) also showed larger within-pair differences (both max and mean Diff_{NN}) (Fig. 5). Correlation coefficients for both comparisons are high ($r = 0.77$ and $r = 0.98$, respectively), but due to low sample size do not reach statistical significance ($P = 0.44$ and $P = 0.13$, respectively). This is interesting

	Dominant frequency	Call duration	Call period	Temperature	Nearest neighbour distance
Snout-vent length	**−0.64**	0.11	0.006	<0.001	−0.09
Dominant frequency		−0.07	−0.06	0.01	−0.11
Call duration			**0.56**	**−0.26**	−0.14
Call period				**−0.50**	−0.04
Temperature					−0.18

Table 2 Correlations between call traits, body size (snout-vent length), temperature and distance to nearest neighbour measured from calling male *Hyla cinerea*. Significant correlations are in bold.
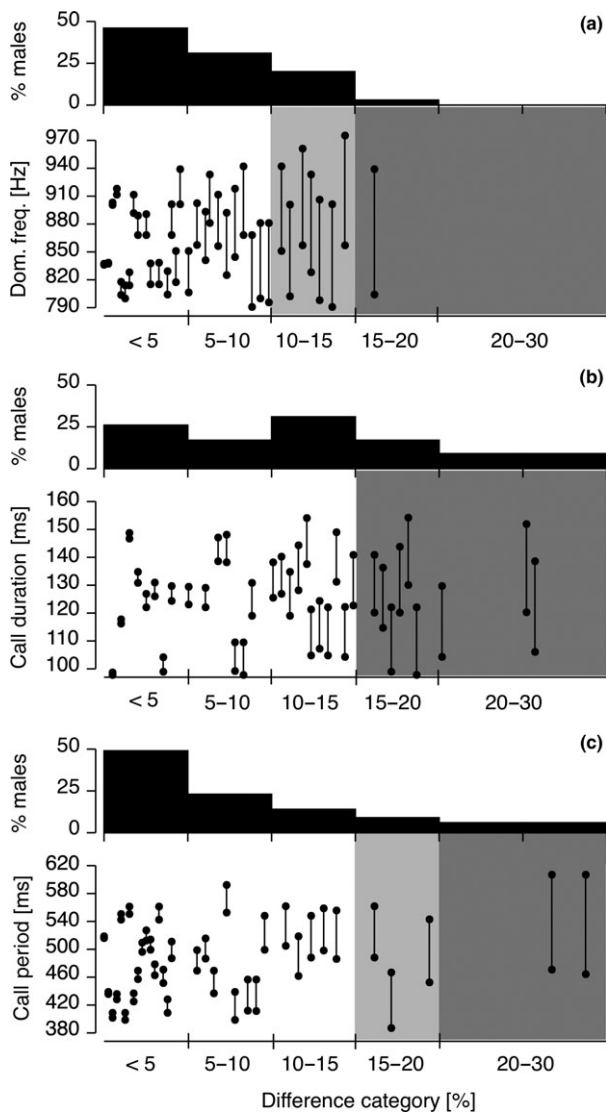
Fig. 3 Call trait differences between nearest neighbour males for (a) dominant frequency, (b) call duration, and (c) call period. Histograms show the distribution of male pairs that differed by the respective magnitude of difference. Graphs below each histogram show the data for individual pairs, sorted by increasing magnitude of difference. Symbols indicate each male's trait value, pairs are represented by symbols connected with lines, and the length of a line indicates the magnitude of difference between the males. Light shading indicates the magnitude of trait difference where > 50% of females discriminate in favour of the more attractive trait value, dark shading indicates the magnitude of trait difference where > 70% of females discriminate in favour of the more attractive trait value (see Fig. 2).

in light of the temperature dependency of these call traits (see Table 2), because it suggests that shared environment is not enough to equalize these call trait differences.

## Estimated mate choice among nearest neighbour males

Based on discrimination thresholds obtained from female choice trials (Fig. 2), males in many nearest neighbour pairs are predicted to be too similar to be differentiated by females (Fig. 3 bottom traces).

Using the relaxed criterion (> 50% of females discriminate in favour of more attractive trait), males in 51% of the nearest neighbour pairs are expected to be chosen by females at random because their trait differences were below threshold. If sufficient differences in any one trait lead to discrimination, then the more attractive male is expected to have higher mating success in 49% of nearest neighbour pairs. If above-threshold differences are considered separately by trait, then 23% of pairs will be discriminated based on dominant frequency differences, 26% on call duration differences and 14% in call period differences. The majority of nearest neighbour pairs differed in only one of the three examined traits; three pairs differed in two traits, and one in three traits.

Using the more stringent criterion (> 70% of females discriminate), males in 69% of the nearest neighbour pairs are expected to be chosen by females at random because their trait differences were below threshold. If sufficient differences in any one trait lead to discrimination, then the more attractive male is expected to have higher mating success in 31% of nearest neighbour pairs. If above-threshold differences are considered separately by trait, then 3% of pairs will be discriminated based on dominant frequency differences, 26% on call duration differences and 6% in call period differences. The majority of nearest neighbour pairs differed in only one of the three examined traits; only one pair differed in two traits, and none differed in all three traits.

Discussion

At the spatial scale at which female green treefrogs likely make their mate choice decisions, the lack of sufficient call trait differences between available males should frequently result in females perceiving them as equally attractive. This has two important consequences for our understanding of mate choice and sexual selection in nature. First, the pattern of realized mating success will likely differ from the pattern of mating success predicted from female preference trials; in particular, the amount of apparent random mating resulting from neighbouring males being too similar may reach a frequency that obscures the existence of female mate choice. Second, sexual selection by female choice should be weakened by locally low call trait variation; selection should still operate, and do so in the *direction* predicted from preference trials, but the *strength* of selection on male traits should be reduced.
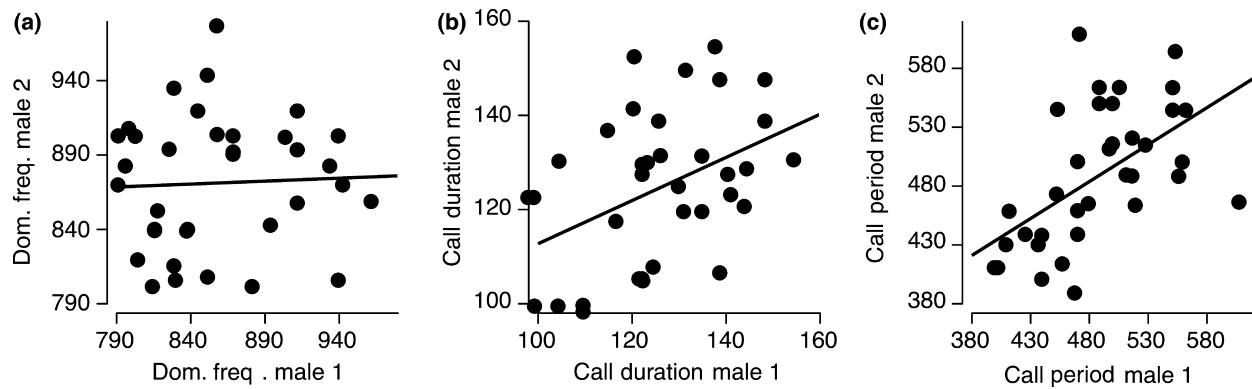
Fig. 4 Comparison of traits between pairs of nearest neighbour males. (a) Dominant frequency was not correlated between neighbouring males ($r = 0.04$; $P = 0.80$), but both (b) call duration ($r = 0.43$, $P = 0.01$) and (c) call period ($r = 0.55$; $P < 0.0005$) were correlated. The axes show the approximate range of trait values found in the population.
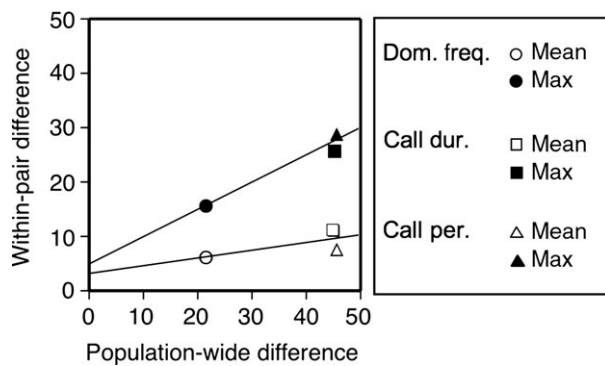
Fig. 5 Traits that show larger population-wide variation ($Diff_{Pop}$) also show larger within nearest neighbour pair differences (both max and mean $Diff_{NN}$).

Although discrimination thresholds have rarely been tested explicitly, several published studies allow extracting data on the trait difference necessary for discrimination. These indicate that discrimination thresholds of the magnitude reported here are fairly typical for female anurans (Oldham & Gerhardt, 1975; Gerhardt, 1991; Doherty & Gerhardt, 1984; Gerhardt & Doherty, 1988; Grafe, 1997; Bosch et al., 2000; Gerhardt et al., 2000; Murphy & Gerhardt, 2000; Rosso et al., 2006; but see some traits with lower thresholds tested in Grafe, 1997; Wollerman, 1998; Gerhardt et al., 2000; Bosch & Márquez, 2005; Bee, 2008). Moreover, as discrimination thresholds are determined under ideal, quiet conditions, yet call recognition in anurans is constraint by background noise (Gerhardt & Klump, 1988; Vélez et al., 2012), females may require even larger display differences for discrimination under natural chorus conditions.

Inferences about the effect of female discrimination thresholds on mate choice and sexual selection, however, can only be made in conjunction with information on the trait differences of the commonly encountered subset of available males. Unfortunately, studies generally only report population-wide display variation, which do not allow for an assessment of how frequently female preferences will translate into higher mating success for the more attractive male. If, as in the present study, average display trait differences are rather small, then a pattern resembling random mating might actually be the most likely pattern of mating success found in the wild. Indeed, patterns of realized mating success are frequently different from the patterns predicted by female preference trials (e.g. Passmore & Telford, 1983; Forester & Czarnowsky, 1985; Gerhardt et al., 1987; Morris, 1989; Smith & Roberts, 2003). The number of males calling (Morris, 1989; Telford et al., 1989), intermale spacing (Dyson & Passmore, 1992) or high levels of chorus-generated background noise (Wollerman & Wiley, 2002) have been implicated as modifying sexual selection under natural conditions. Mating mistakes, that is females not being able to exert choice under complex natural conditions are frequently suggested as the causal agent of the apparent lack of mate choice. By contrast, the present study suggests that seemingly random patterns of male mating success may result from active female choice under conditions of low display variation, where available alternatives are perceived as equally attractive.

Why, as long as there is male trait variation, would there not be selection on females to improve their discrimination ability? A range of factors may affect selection on female discrimination ability, fitness being one of them. The widespread pattern of comparatively moderate discrimination abilities (see above) suggests that mating with a slightly more attractive male does not confer a sufficient increase in fitness benefits, even if mating with an exceedingly attractive male might. In a series of studies with grey treefrogs, Welch and

colleagues compared performance of maternal half-siblings that were sired by males of varying attractiveness and showed that offspring of males with attractive long calls show better performance (i.e. shorter larval period, better post-metamorphic growth; Welch *et al.*, 1998; Doty & Welch, 2001; Welch, 2003). In these studies, the call duration difference between attractive and unattractive sires was 36–50%, which is well above this species' discrimination threshold for call duration (11–20%; estimated from Gerhardt *et al.*, 2000). It would be interesting to determine the call duration difference at which the benefit of mating with a more attractive male disappears, and whether it falls below the female's discrimination threshold.

Patterns of male traits variation

In the present study, mean and even maximum nearest neighbour differences were substantially smaller than population-wide differences. Although this is an intuitive result after the fact, especially if male call traits follow a normal distribution, it highlights the importance of evaluating male trait variation at the scale females are most likely to encounter it. Currently, most studies focus on reporting population-wide trait variation (i.e. Bosch *et al.*, 2000; Gil & Slater, 2000; Zuk *et al.*, 2008), although this is likely not the biologically most relevant measure of trait variation influencing female mate choice. As I am showing here, focusing on population-wide trait variation may overestimate the strength of sexual selection acting on male trait evolution.

Traits that were less variable across the whole population were also less variable between nearest neighbours. For example, call frequency was the least variable trait in both comparisons. This is the call trait for which female green treefrogs show the strongest preference (Gerhardt, 1987; Höbel & Gerhardt, 2003; Höbel, 2010), which suggests that stronger selection by female choice may have decreased variation in call frequency more than in the temporal call traits. Traits under stronger sexual selection often have lower variability (i.e. Rodríguez *et al.*, 2006; Höbel & Gerhardt, 2007; Reinhold, 2011; but see Reinhold, 2009). Reduced variation in spectral compared to temporal call traits may also arise from other fundamental differences between these traits, such as their link to morphology vs. metabolism/behaviour. Call frequency in frogs is determined by the size of the buccal cavity and larynx, which scale with body size (McClelland *et al.*, 1996), while call duration and call period are frequently correlated with energy reserves and/or social competition (e.g. Wells & Taigen, 1986; McClelland *et al.*, 1996; Kime *et al.*, 2004). Frog choruses are highly dynamic, and male frogs are very much attuned to changes in their competitive environment. In particular, temporal parameters like call duration or period can quickly

increase or decrease depending on the performance of near-by rivals (Schwartz *et al.*, 2002; Kime *et al.*, 2004; Höbel, 2015). This socially mediated plasticity increases within-male variation in temporal call traits and may decreases the ability of sexual selection by female choice to erode variation in these traits. The idea that the relative degree of variation in display traits is either more related to female preference strength, or differences in trait architecture (i.e. size vs. condition vs. plasticity) could be tested by examining species in which females show stronger preferences for temporal than for spectral traits, such as the North American grey treefrogs (*Hyla versicolor*, *H. chrysoscelis*) (Gerhardt *et al.*, 2000; Gerhardt, 2005a,b).

Little is known about how nearest neighbour associations are established within anuran choruses (Wells, 2007). Male *Hyla avivoca* treefrogs settle more frequently next to speakers playing attractive compared to unattractive calls (Martínez-Rivera, 2008), which suggests that males can assess the quality of their potential neighbours and seek to be close to attractive ones. In the present study, male *H. cinerea* did not choose their neighbour based on the neighbour's call frequency, because males trying to minimize frequency differences would have been revealed by a significant positive slope in the correlation between the call frequencies of nearest neighbour males, whereas males trying position themselves next to maximally different call frequencies would have been revealed by a negative slope. On the other hand, the within-pair correlation of both call duration and call period suggests that there is either assortative positioning, or that males influence each other's temporal call characters once nearest neighbour associations are established. The latter is more parsimonious, because socially mediated plasticity in temporal call traits is common in anurans (Gerhardt & Huber, 2002). Socially mediated plasticity in calling behaviour has been suggested as a means of maintaining attractiveness under competitive conditions (e.g. Schwartz *et al.*, 2002; Kime *et al.*, 2004). However, unless this plasticity conserves sufficient between-male variation for females to differentiate between males, it may actually equalize mating success for both males. Moreover, in ectotherm animals like *Hyla cinerea* treefrogs, where the call traits influenced by social competition also show temperature dependence, behavioural and ecological factors may combine to further diminish call trait differences between nearest neighbours.

Conclusion

In the present study, female green treefrogs discriminated call trait differences of 10–20%, yet differences between nearest neighbour males were frequently smaller than that. This suggests that hypotheses about sexual selection and signal evolution derived from laboratory preference trials need to be adjusted to

accommodate information about female discrimination thresholds as well as the male display variation that is commonly encountered when females make mate choice decisions in the field. Overall, the direction of selection in the wild should match the one suggested from choice trials, but the strength of selection should be weaker as the proportion of random mate choice due to low display variation increases. It also calls for caution when interpreting data on realized mating success; the lack of a particular pattern of male mating success may not necessarily mean that female preferences are weak or that conditions are too challenging for females to express their preferences (i.e. too noisy, too many predators, etc.). Rather, low display variation at the spatiotemporal scale at which females make mate choice decisions can also result in apparently random mate choice.

Acknowledgments

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