Repeatability of mate choice: the effect of size in the African painted reed frog, *Hyperolius marmoratus*

MICHAEL D. JENNIONS*, PATRICIA R. Y. BACKWELL† &
NEVILLE I. PASSMORE†

**Wildlife Conservation Research Unit, Department of Zoology, University of Oxford,
South Parks Road, Oxford OX1 3PS, U.K.*

†*Department of Zoology, University of the Witwatersrand, Private Bag 3, WITS 2050, Braamfontein,
Johannesburg, South Africa*

Abstract. Female painted reed frogs were offered a choice between artificial advertisement calls differing in frequency. Repeatability of mate choice was assessed by multiple testing of females. When the difference between the stimuli was 400 Hz, almost all females, regardless of size, consistently chose the low frequency call. When the frequency difference between the calls was smaller (200 Hz), females as a group appeared to choose at random. However, individual females seemed to show repeatability of mate choice. There was also a relationship between female size and the number of times they chose the lower frequency stimuli. This suggests that the manner in which females respond to stimuli that differ in frequency may be size-dependent. Larger females may be more sensitive to variation in call frequency, and therefore more likely to express a mating preference for low frequency calls.

Females are capable of expressing mating preferences only if there is phenotypic variation among potential mates. Several recent studies have confirmed that considerable variation in male secondary sexual characters (ornaments) exists, both within (Barnard 1991) and between (Cherry 1990; Ritchie 1992a) populations. Furthermore, significant heritability of sexually selected male traits has been documented in several species (e.g. field crickets, *Gryllus integer*: Hedrick 1988; and great tits, *Parus major*: Norris 1993).

In contrast to the large amount of work on variation in male ornaments, far less attention has been paid to variation in female mating preferences. Although between-population variation in female mating preferences has been demonstrated in guppies, *Poecilia reticulata* (Houde & Endler 1990), and grasshoppers, *Ephippiger ephippiger* (Ritchie 1991), relatively little is known about within-population variation (Kirkpatrick & Ryan 1991). This is an important gap in our understanding of mate choice, because most theoretical models for the evolution of extravagant male traits assume heritable variation in female preferences. ('Mate choice' refers to the observed pattern of mating. 'Mating preferences' are the

underlying psychological or sensory functions that determine which males are most attractive to females. Preferences are usually detected by studies of mate choice.)

One method of investigating the extent of within-population variation in female preferences is to determine the repeatability of mate choice (Boake 1989; Gerhardt 1992). Repeatability provides an index of the level of phenotypic variation among females. The degree of repeatability of mate choice also sets an upper limit to the heritability of female preferences. For example, if females show no repeatability in their choice of mates, then there is presumably no variation in mating preferences between females. Observed variation in mate choice can therefore be attributed to within-individual variation. Conversely, repeatable patterns of mate choice that differ between females suggest that female mating preferences vary. Of course, these conclusions rest on the assumption that mating preferences are being expressed and are not constrained by contingent environmental factors.

In the study described here we offered female painted reed frogs a choice between high and low frequency advertisement calls, and low and

medium frequency calls, and documented the repeatability of their responses. Recent work has shown that female frequency tuning curves in anurans may be related to body size (Keddy-Hector et al. 1993). We therefore also looked at the potential importance of female body size as a correlate of frequency preference that might account for within-population variation in mate choice.

METHODS

Phonotaxis experiments were conducted between 22 December 1992 and 29 January 1993 in Mtunzini, South Africa (28°51′S, 31°46′E). Gravid females were captured at nearby ponds and transported to the testing site (less than 3 km away). All females were tested on the night of capture, and released the following night at the ponds. They were tested in an outdoor arena (3 × 3 m) illuminated by a red fluorescent light suspended 1·8 m above the arena. Ambient temperature ranged from 18 to 24°C. The broadcast stimuli used in the two-choice phonotaxis experiments were synthetic advertisement calls identical with respect to the extent of sweep (500 Hz), duration (80 ms) and rise and fall time (10 and 25 ms, respectively). They differed only with respect to frequency. The low frequency (2·9 kHz) stimuli had a sweep excursion of 2650–3150 Hz. The middle frequency (3·1 kHz) stimuli had a sweep excursion of 2850–3350 Hz, and the high frequency (3·3 kHz) stimuli had a sweep excursion of 3050–3550 Hz. The dominant frequencies of these stimuli fall within the natural range of the study population (Dyson & Passmore 1988). The sound pressure level of stimuli was set at 104 dB (peak) at 50 cm, which is the mean intensity of a natural call at this distance. Measurements were made using a Brüel & Kjaer 2230 sound level meter and a Brüel & Kjaer type 4165 microphone. Calls were played alternately from each speaker at the mean natural call rate of 60 calls per min. Calls were broadcast using a Sony TCD-5M tape-recorder, a Klein & Hummel SB 280/II stereo amplifier and two Philips AD50600 5-inch diameter loudspeakers. The loudspeakers were 3 m apart, 180° from each other and equidistant from the release container. The observer sat to one side of the arena, equidistant from the speakers. Females were left in the release container for 3 min prior to the first

trial, and for a minimum of 2 min between subsequent trials. For further details on the phonotaxis set-up and sonagrams of *H. marmoratus* see Figure 1a in Dyson & Passmore (1988).

In the first experiment the choice was between the 2·9 and 3·1 kHz stimuli (2·9/3·1 experiment). Each female was tested in four trials, and data from a female were discarded if she failed to respond in any of the trials. Twenty females were tested successfully; three failed to complete all four trials. In the second experiment the choice was between the 2·9 and 3·3 kHz stimuli (2·9/3·3 experiment). Sixteen females were tested successfully, one failed to complete all four trials. Twelve females were used in both experiments. Six (50%) were first tested in the 2·9/3·1 experiment. Stimuli were played from the same speaker for a given female, and alternated between females. There was no end bias. We scored a positive response if the female touched the speaker or approached to within 10 cm of the speaker. Females were classified as unresponsive if they remained immobile for more than 5 min, or attempted to escape from the arena. Snout–vent length (SVL) of females was measured to the nearest 0·1 mm using vernier callipers. In the 2·9/3·3 experiment three females escaped before being measured.

We analysed the number of responses to each of the stimuli in the two-choice tests using exact binomial probabilities to test for a directional preference. To test for repeatability of choice, we compared the distribution of the number of responses per female to one of the stimuli to binomial distributions using chi-squared goodness-of-fit tests and the log-likelihood ratio test. Probability values for the binomial distributions were calculated from the responses in the first trial of each experiment. In some comparisons, categories were lumped to ensure that the smallest expected value was greater than one (Zar 1984). The Spearman rank correlation coefficient was calculated with correction for tied ranks in the 'proportion of responses'. All tests are two-tailed. Values are presented as $\bar{X} \pm$ SD.

RESULTS

In the 2·9/3·1 experiment there was no significant preference for either stimuli in any trial (binomial exact, $P > 0.54$; Table I). Even if each trial was assumed to be statistically independent, thereby

Table I. Response of females to 2·9 kHz versus 3·1 kHz calls in first and subsequent trials

Trial	Number of responses to		Binomial probability (two-tailed)
	2·9 kHz	3·1 kHz	
First	11	9	0·82
Second	11	9	0·82
Third	12	8	0·54
Fourth	12	8	0·54
Total	46	34	0·22

Table II. Response of females to 2·9 kHz versus 3·3 kHz calls in first and subsequent trials

Trial	Number of responses to		Binomial probability (two-tailed)
	2·9 kHz	3·3 kHz	
First	14	2	0·004
Second	15	1	0·0005
Third	14	2	0·004
Fourth	15	1	0·0005
Total	58	6	<0·00001

increasing the sample size to 80, there was still no preference for either stimulus (binomial exact, $P=0.22$). There are two possible explanations for this random response. First, females may have shown no individual preference for either stimulus. Second, individual females may have shown different preferences with some females preferring the higher frequency, and others the lower frequency stimulus. If the random response is due to a lack of female preference for either stimulus, we expect the distribution of the number of responses per female to one of the stimuli to follow a binomial distribution (where $P=Q=0.5$; Fig. 1). However, when we examined the repeatability of female choice we did not find this to be so (log-likelihood goodness-of-fit test, $G=11.02$, $df=4$, $P<0.05$; Zar 1984, pp. 52–53). For chi-squared tests, it is recommended that no expected value be less than 1, and no more than 20% of

Figure 1. The frequency distribution of the number of responses per female *H. marmoratus* to the 2·9 kHz stimuli in the 2·9 kHz versus 3·1 kHz experiment ($N=20$ females). The expected values are based on the binomial distribution when $P=0.5$.

expected values be less than 5. The data can be pooled in two ways; pooling the 4 and 3 response classes, or the 0 and 1 response classes. In the former case, the distribution is almost significantly different from the binomial distribution ($\chi^2=7.13$, $df=3$, $P=0.068$); in the latter case it is significantly different ($\chi^2=12.89$, $df=3$, $P<0.01$). Sixteen of the 20 females (80%) chose the same stimulus three or more times, compared to an expected 63%, and eight of the 20 females (40%) were consistent in their choice in all four trials compared to an expected 13%. If the expected distribution is calculated using the initial proportion of responses to the 2·9 kHz stimuli to predict the value of P, then $P=0.55$ (11/20). Again, the observed distribution almost differed significantly from the predicted binomial distribution ($\chi^2=7.47$, $df=3$, $P=0.058$). These results show that females were not choosing between the stimuli at random and that more females than expected repeatedly chose the same stimulus.
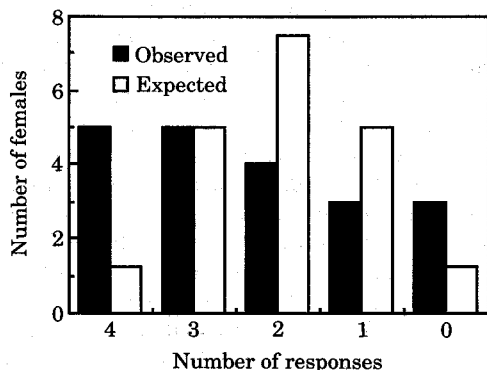
In the low (2·9 kHz) versus high (3·3 kHz) frequency experiment all trials revealed a significant female preference for the low frequency stimuli (binomial exact, $P<0.004$; Table II). If the initial response to the stimuli reflects a preference for the low frequency call that does not differ between females, the distribution of the proportion of responses per female to the preferred stimuli should fit a binomial distribution, where P is calculated using the initial proportion of responses to the preferred stimuli (hence $P=14/16=0.875$). The observed distribution did not differ from the predicted binomial distribution ($\chi^2=1.83$, $df=2$, $P=0.40$). Hence this result is consistent with a situation where all females show a strong preference for the low frequency call.

Female Size

We tested to see whether female size might account for the direction of the bias in female mate choice. In the 2·9/3·1 experiment the females that initially chose the 2·9 kHz stimuli and those that chose the 3·1 kHz stimuli did not differ in size (2·9 kHz: $30·9 \pm 1·0$ mm, $N=10$; 3·1 kHz: $30·4 \pm 1·5$ mm, $N=8$; Mann–Whitney U-test, $z=0·667$, $P=0·50$). However, when we repeated this comparison using data for all four trials, we found that females showing a bias towards the 2·9 kHz call were significantly larger than those showing a bias towards the 3·1 kHz call (2·9 kHz: $31·0 \pm 1·05$ mm, $N=10$; 3·1 kHz: $29·7 \pm 1·2$ mm, $N=6$; Mann–Whitney U-test, $z=2·115$, $P=0·034$). There was also a marginally significant correlation between female size (SVL) and the number of times a female chose the 2·9 kHz stimuli ($r_S=0·44$, $N=18$, $P=0·07$). There was no relationship between temperature and the number of times females choose the 2·9 kHz stimuli ($r_S=0·02$, $N=18$, $P=0·91$), even when SVL was controlled for (Kendall partial rank-order correlation coefficient, $T_{xyz}=0·12$, $P>0·5$).

In the 2·9/3·3 experiment, there was no size difference between females that chose the 2·9 kHz stimuli in all four trials ($30·8 \pm 1·2$ mm, $N=9$), and those that responded once or more to the 3·3 kHz stimuli ($30·3 \pm 1·5$ mm; $N=4$, Mann–Whitney U-test, two-tailed, $z=0·46$, $P=0·64$). There was also no correlation between female size (SVL) and the number of responses to the 2·9 kHz stimuli ($r_S=0·22$, $N=13$, $P=0·44$). However, the sample size for females responding to the 3·3 kHz stimuli is small ($N=4$), and this test therefore has little statistical power.

DISCUSSION

In anurans, low frequency calls may act as signals of male quality because there is usually a negative correlation between advertisement call frequency and male size (Ryan 1985). Several studies have shown a female preference for intermediate call frequencies resulting in stabilizing selection (Gerhardt 1982), but directional preferences are always for lower frequency calls (Ryan & Keddy-Hector 1992). However, the absence of directional mate choice at the population level does not exclude the possibility of female preferences for

extravagant ornaments. For example, there may be continuous variation in female preferences with only some females preferring larger ornaments and/or there may be a relatively invariant female preference for larger ornaments, but environmental conditions, contingent aspects of a female's phenotype or inter-sexual competition may mask these preferences.

In most phonotaxis studies of mate choice each female is tested once per experiment. In our study the first trial responses of females offered a choice between the 2·9 and 3·1 kHz stimuli indicated that there was no preference for the lower frequency call at the population level. However, multiple testing of females suggested that they were not choosing stimuli at random. More females than expected consistently chose the same stimuli. The possibility of this occurring in anurans was previously raised by Ryan et al. (1992), but was not tested directly by determining the repeatability of female choice. Gerhardt (1991, 1992) has noted that there is a general lack of evidence for variability in female mating preferences in frogs, although he found that nine of 12 female treefrogs, *Hyla cinerea*, chose the same stimulus in at least 75% of tests, when the choice was between a conspecific and hybrid call (Gerhardt 1974). Ritchie (1992a) did not find repeatable female preferences for calls that varied in syllable number within a population of bushcrickets, *E. ephippiger*. To our knowledge, this is the first study of an anuran suggesting repeatability of female choice when presented with two conspecific stimuli.

Why does this variation in female mate choice exist? Our data do not support the usual assumption that repeatable mate choice reflects between-female variation in mating preferences (cf. Boake 1989). Although females showed repeatable responses when offered a choice between the 2·9 and 3·1 kHz stimuli (suggesting within-population variation in mating preferences), there was an almost unanimous preference for the low frequency call when the choice was between 2·9 and 3·3 kHz stimuli. This absence of a directional preference in the 2·9/3·1 experiment fits with the commonplace observation that, at least at the population level, directional preferences are not seen when the magnitude of the frequency difference between stimuli is small (e.g. Ryan 1985; Morris & Yoon 1989). What is interesting about our results, however, is that female body size may affect the ability of females to distinguish between

stimuli. When the frequency difference between stimuli was small, larger females were more likely to respond to the lower frequency stimuli. Female body size, or a correlate thereof, may thus act as a constraint on the ability of females to distinguish between calls that differ in frequency. We are unaware of other studies documenting a similar effect of female phenotype on the ability to distinguish between stimuli.

There is an alternative explanation for our results, which does not invoke differences in discriminatory ability. Larger females may be preferentially attracted to 2·9 kHz, or even lower frequency calls, while smaller females may prefer calls intermediate between 2·9 and 3·1 kHz. This would explain the almost unanimous preference for 2·9 kHz when the choice was 2·9 kHz versus 3·3 kHz (because 2·9 kHz is closer to the preferred call of smaller females); and the difference between smaller and larger females when the choice was between 2·9 and 3·1 kHz. However, other phonotaxis studies in *H. marmoratus* weaken this explanation. When females are offered a choice between 2·7 and 3·3 kHz most ($N=15$ of 18) showed a strong preference for the 2·7 kHz stimuli (M. L. Dyson, personal communication), suggesting that there is a directional frequency preference which is not size-dependent.

At the proximate level, it is unclear why larger females should be more sensitive to differences in call frequency than smaller females. Neurophysiological work in female cricket frogs, *Acris crepitans*, has shown a negative relationship between the frequency tuning of the basilar papilla and body size (Keddy-Hector et al. 1993). In *A. crepitans*, females that initially chose a lower frequency call in phonotaxis experiments were significantly larger than those that chose a high frequency call (Ryan et al. 1992). In our study a similar relationship between size and female call preferences was apparent when the data from all four trials were used (emphasizing the importance of multiple testing). The neurophysiological work on tuning curves in *A. crepitans* suggests that size-based variation in female mate choice is due to a relationship between frequency preferences and body size. However, our data from *H. marmoratus* suggest an alternative explanation for this type of size-based relationship. Variation in mate choice may also be caused by body-size dependent variation in the ability to distinguish between stimuli.

This study and that of Ryan et al. (1992) show that female body size in anurans may influence mate choice. Relatively few studies have been conducted to determine the heritability of female mating preferences (but see Majerus et al. 1986; Sappington & Taylor 1990; Ritchie 1992b), but these are needed to confirm that variation in choice is due to heritable variation in preferences. By performing breeding experiments, other explanations for variation in mate choice can also be investigated, including those based on variation in body size. In *A. crepitans*, inter-population variation in call-tuning allometry cannot be accounted for solely in terms of body size, suggesting that additional factors also play a role in the evolution of female frequency preferences (Ryan & Wilczynski 1988). The extent to which a relationship between size and female auditory tuning can explain variation in female mate choice in *H. marmoratus* is unknown. However, regardless of whether female preferences are heritable, or whether they are contingent on body size, the continued presence of phenotypic variation between females in their ability to distinguish between males, or in their actual preference for specific call parameters is important because it may act to maintain heritable variation in male calls despite the presence of directional selection.

Previous work has shown that there is a significant relationship between male size and call frequency in *H. marmoratus* with larger males having lower frequency calls (Dyson & Passmore 1988). Given that larger females seem to be more likely to respond to low frequency calls, this should lead to positive assortative mating with respect to size within natural choruses. However, in a recent study of natural mating patterns in *H. marmoratus*, which involved more than 1000 marked males, there was no evidence for positive assortative mating on the basis of size, or of a large-male mating advantage (Dyson et al. 1992). These findings emphasize the limitations of laboratory experiments of female choice. Preferences documented in the laboratory may have no effect in the field, possibly because of the increased acoustic complexity of the natural situation (Gerhardt 1992), male–male competition, less phenotypic variation between males in the field (Cherry 1990), and other costs or constraints that make fine-scale discrimination between calls unlikely.

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