



Plasticity in female mate choice associated with changing reproductive states

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Flexibility in female mate choice occurs over a range of timescales. We examined this plasticity over the course of a single breeding cycle using phonotaxis tests that assay acoustic-based mating preferences of female túngara frogs, *Physalaemus pustulosus*. Tests were performed throughout three reproductive stages: unamplexed, amplexed and postmated. We tested whether the approach of the time at which a female needs to release her eggs (i.e. amplexed stage) influences her mate decisions. We examined three aspects of female mate choice behaviour: receptivity, permissiveness and discrimination. We considered a female receptive if she accepted a conspecific male's call, permissive if she accepted a call that was less attractive than a conspecific call and discriminating if she maintained her preference for a 'whine-chuck' over a 'whine'. Our results show that the proportion of females responding receptively and permissively peaks during the amplexed stage. Individual females also increase receptive and permissive responses in the amplexed stage. Furthermore, time to respond to mate signals is lowest in the amplexed stage. The increase in permissive mate decisions was not associated with a decrease in the probability of expressing discriminatory behaviour. We suggest that the increase in permissive mate choice is due to a decrease in female choosiness, that is, a lowering of her threshold for accepting unattractive calls, as her receptivity increases. This study provides empirical support for theoretical models predicting that females will reduce their threshold criteria for choosing mates as critical time points approach.

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For the sake of convenience, mate choice studies treat female choice as if it were static. Recent studies, however, report that females are flexible in their mate decisions over the course of their lifetime (Kodric-Brown & Nicoletto 2001; Moore & Moore 2001), throughout the breeding season (Qvarnström et al. 2000; Veen et al. 2001) and even throughout a single reproductive cycle (Lea et al. 2000). Theoretical models of mate choice behaviour suggest that it is important for females to be flexible in their mate decisions because of constraints that may be imposed upon the female as she is actively searching for a mate (Real 1990). Such constraints may arise from both intrinsic and extrinsic factors. Extrinsic factors, such as high predation risk, can increase the cost of mate sampling (Crowley et al. 1991; Endler & Houde 1995; Rand et al. 1997), thereby influencing a mate-searching female to use

sampling strategies that have reduced costs, such as mate copying (reviewed in Pruett-Jones 1992). Intrinsic factors, such as the approach of the time at which the breeding season will end or the time at which egg deposition is imminent, can cause time constraints on mate-searching females. As these critical times approach, the female may be faced with a trade-off in which she will have to decrease the strength of her mate preference, thereby expressing plasticity in her mate choice, or risk losing the opportunity to fertilize her eggs.

Although theoretical models predict the appearance of flexibility in mate decisions under constraints and recent empirical studies have tested these predictions, no study systematically examines different aspects of mate choice behaviour to determine how each contributes to overall flexibility in mate choice. This study examines three aspects of mate choice behaviours, receptivity, permissiveness and discrimination. We consider receptive behaviour to be a response to any conspecific call. Receptivity can also be thought of as indicating a female's motivation to mate so that an increase in this behaviour increases the

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probability that she will fertilize her eggs. A permissive mate choice is one in which a female responds to a mate signal that is normally unattractive, perhaps even at the minimum threshold for a female's response; a response to this signal will increase the female's probability of mating, but not with her preferred phenotype. There are two alternative explanations for the occurrence of a permissive mate choice. First, it is possible that the ability of the female to discriminate or discern the difference between mate signals has been reduced (Gerhardt 1987). Alternatively, the female's ability to discriminate amongst mate signals may remain intact but she may instead lower her threshold for accepting signals (i.e. become less choosy). This may reflect a reduction in the energy and/or time she is willing to invest in mate sampling (Jennions & Petrie 1997). We can discern between these alternative hypotheses by testing whether a female that responds to a mate signal with a reduced quality when she is given no other choice also maintains her discriminatory response to the preferred signal when it is readily available, indicating that her discriminatory ability has not been reduced.

The objectives of this study were two-fold. First, we examined whether mate choice behaviours that increase the probability of fertilization, such as receptivity and permissiveness, increase under time constraints caused by the approach of the time at which the female must oviposit. Second, we examined whether an increase in permissive mate choice was associated with either a decrease in the female discrimination or a decrease in female choosiness. An association between permissive mate choice and a decreased response to the preferred signal when it is available would support the conclusion that a decrease in discrimination contributes to permissiveness. Alternatively, an increase in permissive mate choice while discrimination in favour of the preferred call is maintained would support the conclusion that a decrease in female choosiness contributes to permissive mate choices.

Study System

We examined mate choice plasticity using anuran amphibians because anurans frequently serve as model systems for investigating basic principles of mate choice behaviour (Ryan 1985; Ryan & Rand 2001; Gerhardt & Huber 2002). Most female anurans base mate choice decisions almost entirely on the advertisement call produced by the male (Wells 1977; Rand 1988). Their behaviour is relatively stereotyped and can be readily elicited in laboratory phonotaxis tests. Therefore, we used phonotaxis tests to assay the mate choices of female túngara frogs, *Physalaemus pustulosus*, a Neotropical species that has been the subject of a long series of investigations into sexual selection and mate choice. It is well understood how variation in male signals within túngara frog populations can influence female preferences (Ryan 1980, 1985, 1997; Rand & Ryan 1981; Ryan & Rand 1990, 2001; Rand et al. 1992; Wilczynski et al. 1995, 1999) as well as how variation in auditory tuning and signals both within and between *Physalaemus* species can influence female preference (Ryan et al. 1990; Ryan & Rand

1993a, Ryan and Rand, 1999; Wilczynski et al. 2001). The males of this species produce an advertisement call that is a frequency-modulated 'whine', to which they may add one or more 'chucks' at the end. The whine is sufficient to elicit phonotaxis, but female túngara frogs significantly prefer whines with adornments added to the end (Rand & Ryan 1981; Rand et al. 1992; Ryan & Rand 1990, 1993b). When presented with a whine versus a 'whine-chuck', female túngara frogs will strongly prefer (i.e. will preferentially show phonotaxis towards) the whine-chuck. Females will show phonotaxis to a whine when it is presented by itself or paired against a noise burst, and will show a strong preference for their conspecific whine over the whines of other *Physalaemus* species. When presented only with calls that hybridize elements of conspecific and heterospecific whines, female túngara frogs will express some degree of phonotaxis towards the artificial hybrid call. In this study we used a synthetic, artificial hybrid call that had a general response rate of about 25% (Ryan et al. 2003), indicating that it is a call that is less attractive than the females' preferred call, the whine-chuck. We used this call as a stimulus to assess changes in female permissiveness.

As for many other amphibian species, female túngara frogs have distinct stages of reproductive behaviour during the breeding cycle. The females ovulate and mate several times within a season during periods of approximately 4–6 weeks (Davidson & Hough 1969; Ryan 1985). During this time they move into breeding aggregations where males are calling, then mate, and leave until another clutch of eggs develops and is ready for fertilization. We repeatedly tested individual females as they progressed through a single reproductive cycle. This design allowed us to assess whether mate choice plasticity occurs in the túngara frog on a timescale as short as a single reproductive cycle.

METHODS

Reproductive Stages

Phonotaxis tests were repeated with individual females as they progressed through three reproductive stages: unamplexed, amplexed and postmated (Fig. 1). Females in the unamplexed stage were located at or near breeding

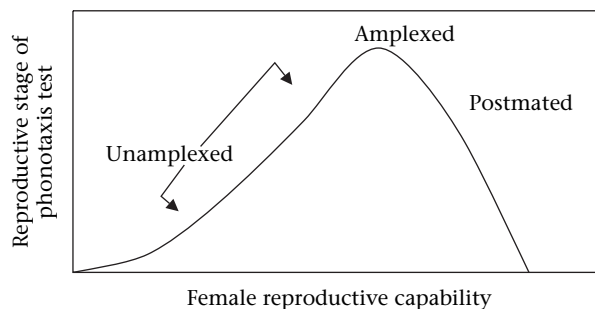


Figure 1. The three reproductive stages in which female receptivity, permissiveness and discrimination of conspecific mate signals were tested.

ponds but before they had chosen a mate. Although Ryan (1985) reported that females come to the breeding pond only on the night they mate, it was still not possible for us to determine whether these females had already mated, were a few days from mating or were within hours of mating. Therefore, this group is considered a heterogeneous group that may be composed of females in different reproductive condition. We examined this possibility by dividing females in the unamplexed stage into two groups based on the amount of time required to oviposit after they completed phonotaxis tests in the unamplexed stage (range 0.5–100 h). These groups included: unamplexed females that mated within 24 h of testing ($N = 4$) and unamplexed females that mated more than 24 h after testing ($N = 10$). Fisher's exact test was used to compare the responses of unamplexed females in the receptivity and permissiveness phonotaxis tests to determine whether the females' responses were influenced by their readiness to oviposit.

When the female has chosen a male, she will approach him and allow him to dorsally mount her and clasp her with his forelimbs. This is known as amplexus and this was the next reproductive stage we examined. Amplexus behaviour brings the cloacae nearly into contact as the two frogs simultaneously release gametes. Male and female frogs will remain in amplexus for several hours. Once the female enters into amplexus and allows the male to clasp her, this can serve as a behavioural indicator that the female is near the point at which she will release her eggs. In order to remeasure the phonotactic responses of the same females that were tested in the unamplexed condition, we held each subject in a 10-gallon (37.85-litre) aquarium with water and a thick layer of leaf litter, which was used as shelter. A single male was also placed in the aquarium. We tested the female's mate choices again when she emerged from the leaf litter and allowed the male to amplex her, which provided us with an indication that she would soon deposit eggs. During the interim, natural mate choruses were broadcast from 1900 hours to approximately 0300 hours and all subjects were fed termites every other day until their release. It took an average \pm SD of 3.3 ± 2.36 days (range 1–9 days) for females to enter into amplexus with the male that was provided to them. Variation in female attractiveness to the male did not appear to impact the time in which females entered into amplexus, because a male would amplex a female, even an unreceptive female, whenever the opportunity presented itself.

Finally, we placed the female back into the aquarium with the same male and allowed mating to proceed. Following mating, the female deposits her eggs in a foam nest (Heyer & Rand 1977), then the pair leaves with no further investment. In the present study, it took an average \pm SE of 1.04 ± 0.72 days (range 0–3 days) for the female to enter into amplexus again and release her eggs. The time at which the female oviposited was recorded. In the postmated stage, we tested females' phonotaxis response approximately every other night until they became unreceptive to conspecific mate signals ($\bar{X} \pm \text{SD} = 3.6 \pm 3.7$ days). Some subjects did not become unreceptive during the postmated stage.

Field Collections

Female túngara frogs were collected in Gamboa, Panama during June and July of 2001. Most female *P. pustulosus* were captured while unamplexed ($N = 31$) between 1930 and 2200 hours, but some females were captured in amplexus ($N = 13$). Snout–vent lengths of females captured while unamplexed ($\bar{X} \pm \text{SD} = 32.16 \pm 1.46$ mm) were significantly larger than those of females captured during amplexus (30.99 ± 0.59 mm; $t_{42} = 2.78$, $P < 0.005$), and 61% of the unamplexed females subsequently mated. This indicates that females in the unamplexed group can be considered sexually mature. Females were brought into the laboratory at the Smithsonian Tropical Research Institute in Gamboa for phonotaxis tests after they were captured. After each female completed all phonotaxis tests she received a unique toe clip number and was returned to the site at which she was captured. We followed the recommended toe-clipping guidelines of the Applied Ecology Research Group. Many toe-clipped females were recaptured; however, recaptured females were not used for further phonotaxis tests in this study.

Phonotaxis Chamber and Phonotaxis Experiments

The testing chamber measured 1.8×2.7 m and was equipped with acoustic foam on the walls to reduce acoustic reverberation. Two ADS L2000 speakers were placed 2.7 m apart at equal distances from the centre of the chamber. The 10-cm point was marked completely around each speaker. The peak intensity of the acoustic stimulus was set at 82 dB SPL (re. 20 μPa) in the centre of the chamber where the female was released. The phonotaxis chamber contained a video camera and an infrared light so that behavioural observations could be made from outside the chamber.

Phonotaxis tests began at approximately 1900 hours and ended at approximately 0800 hours. At the start of the phonotaxis test each subject was placed in the centre of the chamber under a funnel for 3 min. During this time the acoustic stimuli were broadcast antiphonally from each speaker with a 0.5-s delay between presentations. The side on which each stimulus was presented was alternated to control for side bias. Once the funnel was lifted, the female was given 15 min to respond to either stimulus. A response was recorded if the female came within 10 cm of a speaker. If she remained stationary for at least two consecutive minutes, failed to move from the release site within 5 min, or did not approach a speaker within 15 min, she was recorded as unresponsive to the acoustic stimuli.

Each subject completed three consecutive phonotaxis tests per reproductive stage. The first and third phonotaxis tests were used to measure receptivity. In each test, the female heard a conspecific whine from one speaker and a conspecific whine-chuck from the opposing speaker. A response to either of these stimuli was sufficient to label the female as receptive, however, the female needed to respond in the first and last phonotaxis tests to be labelled

as receptive. Therefore, this test only measured response or no response to any conspecific call. Females that did not respond in either the first or last test were recorded as unreceptive. Females that approached a speaker in only one of the two tests were also considered nonreceptive, because we could not be sure that an apparent response in only one test indicated receptivity or was simply a random movement towards one of the speakers. All subjects completed the remaining phonotaxis tests regardless of their receptive state. The next phonotaxis test was used to measure female permissiveness. During this test, the female heard a synthesized artificial hybrid whine from one speaker and white noise of equal duration and amplitude from the opposite speaker. This synthesized artificial hybrid call had previously been determined to elicit a 25% response rate from female túngara frogs, which indicates that it is a less attractive advertisement call than the conspecific mate call (Ryan et al. 2003). Females that approached the speaker broadcasting the hybrid whine were recorded as responding permissively, whereas females that did not approach the speaker were recorded as nonpermissive responders. We used the last phonotaxis test to measure receptivity and discrimination. Females that consistently responded to the whine-chuck in both the first and last phonotaxis tests were considered discriminatory responders, whereas females that were not consistent in their choice of the whine-chuck were considered nondiscriminatory responders.

We also recorded the latency to respond (time from raising of the funnel to the female arriving within 10 cm of a speaker) in each of the tests. Subjects that did not respond received a latency score of 900 s, which was the maximum time allowed for each female to make a choice.

All stimuli were synthesized on a Dell computer with unpublished software produced by J. Schwartz. Previous phonotaxis tests using synthesized conspecific calls versus natural calls showed that female *P. pustulosus* do not discriminate between the two call types, indicating that the synthetic call captures the salient features in the conspecific call that are necessary to elicit phonotaxis behaviour (A. S. Rand & M. J. Ryan, unpublished data). The synthesized call is based on the average signal parameters and has been used in a large number of phonotaxis experiments with this species, providing a baseline of response. The artificial hybrid call was a synthetic call with parameters that were intermediate between the calls of *P. pustulosus* and *P. enesefae* (see Ryan et al. 2003).

Statistics

The number of females that completed the phonotaxis tests in each stage varied: unamplexed ($N = 31$), amplexed ($N = 34$) and postmated ($N = 30$). The total number of subjects in each group included females that repeated phonotaxis tests in two or three reproductive stages and some females that completed tests in only a single reproductive stage. Therefore, we used descriptive statistics to examine the behavioural pattern for all of the females tested (Table 1).

Table 1. Pattern of receptive and permissive behaviours for all females tested throughout a single reproductive cycle

| | Unamplexed | Amplexed | Postmated |
|-----------------------------------|------------|----------|-----------|
| Receptivity | | | |
| % Response | 29 | 53 | 20 |
| Mean \pm SD time to respond (s) | 616.72 | 404.04 | 673.33 |
| | 323.41 | 339.38 | 280.16 |
| Permissiveness | | | |
| % Response | 16 | 35 | 16.7 |
| Mean \pm SD time to respond (s) | 785.83 | 618.18 | 786.76 |
| | 273.82 | 390.12 | 279.41 |
| <i>N</i> | 31 | 34 | 30 |

Results of repeated measures comparisons supported this general pattern of female receptivity and permissiveness. The changes in response frequencies throughout the three reproductive stages were due to changes in the responses of individual females (see Table 2).

Responses of some females that were collected in the unamplexed stage were not measured in the amplexed stage, because they released eggs rapidly once they were amplexed, and responses of some females that were collected in the amplexed stage were only measured in the amplexed and postmated stages. Consequently, there were 37 females that completed the phonotaxis tests in at least two reproductive stages and only 11 females that completed the phonotaxis tests in all three reproductive stages. Therefore, we analysed the data using all possible pairwise comparisons, which included: females that completed both the unamplexed and amplexed stages ($N = 20$), females that completed both the amplexed and postmated stages ($N = 22$), and females that completed both the unamplexed and postmated stage ($N = 16$).

Proportion of Responsive Females

We used a chi-square goodness-of-fit test to examine whether the proportion of females that responded in the receptivity and permissiveness tests differed significantly between reproductive stages. The number of responses in one reproductive stage was used as the expected response frequency and the number of responses from the same females in a different reproductive stage was used as the observed frequency. This allowed us to test females against their own responses. We used the amplexed reproductive stage as our expected group whenever possible. Separate analyses were done for receptivity and permissiveness tests. The alpha value was set at 0.05.

Responses of Individual Females

We also used a chi-square test to examine whether individual females significantly decreased their responsiveness in the receptivity and permissiveness tests between stages. Females that were responsive during the receptivity and/or permissiveness test in the first reproductive stage

were examined to determine whether they were still responsive during these phonotaxis tests in the second reproductive stage. This analysis was conducted as described above. In addition, we used a paired samples *t* test to examine whether the female's response time during the receptivity and permissiveness phonotaxis tests was significantly different between stages. We compared females' latency to respond during the receptivity tests by averaging response time in both the first and last phonotaxis test for each group of females. Females that did not respond received a score of 900 s, which was the maximum time allowed for each female to make a choice.

Discrimination

Because we assessed discriminatory responses of each female that completed one reproductive stage, we used the total number of females responding to the whine-chuck in both the first and the third phonotaxis tests (conspecific call tests) to determine the percentage of discriminatory response within each reproductive stage. We used a Fisher's exact test to determine whether the proportion of unamplexed or postmated females displaying discriminating responses differed from the proportion of females displaying discriminating responses in the amplexed stage.

Association between Receptivity and Permissiveness

We used two analyses to examine whether more motivated females, as measured by their latency to respond to a conspecific signal, were also more likely to respond permissively. First, we divided the total number of amplexed females into permissive ($N = 12$) and nonpermissive ($N = 22$) responders, then compared each group's latency to respond to conspecific mate calls using a Student's *t* test. In addition, we examined whether the time to respond to a conspecific call in the receptivity test significantly explained the variation in the time to respond to an artificial hybrid call in the permissiveness test using simple linear regression. Nonresponses were given a score of 900 s.

Latency to Respond to Mate Signals and Time to Oviposit

We recorded the time from the last phonotaxis test to oviposition for 25 females. Periodic checks (0.5–5 h) were made to note whether the female released eggs. Because we did not have a continuous method for checking the time of egg release, we used an analysis in which we divided females according to whether they required more ($N = 11$) or less ($N = 14$) than the median time to oviposit. The median time was 14.5 h after their last phonotaxis test. We compared the latency to respond in both the receptivity and permissiveness phonotaxis tests using a Student's *t* test. All alpha values were set at 0.05 for these comparisons. Values are given as means \pm SD unless stated otherwise.

RESULTS

Receptivity

Descriptive statistics for all females that completed receptivity phonotaxis tests in at least one stage showed that the time to respond to conspecific calls decreased from the unamplexed to the amplexed stage and increased again in the postmated stage. Similarly, the frequency of response to these calls increased from the unamplexed to the amplexed stage and declined again in the postmated stage (Table 1).

Proportion of Receptive Females

Among females that completed both the unamplexed and amplexed reproductive stages, three females responded in both conspecific call tests in the unamplexed stage. When those same females entered into amplexus, nine females responded each time they were tested with a conspecific advertisement call ($N = 20$; $\chi^2_1 = 7.27$, $P = 0.007$; Fig. 2a); thus, the proportion of females that expressed receptivity at these two stages differed significantly. Fourteen females responded to the conspecific calls while in the amplexed stage but when those same females entered the postmated stage, only four responded ($N = 22$; $\chi^2_1 = 19.64$, $P < 0.001$; Fig. 2a), again indicating that the proportion of receptive females changed significantly

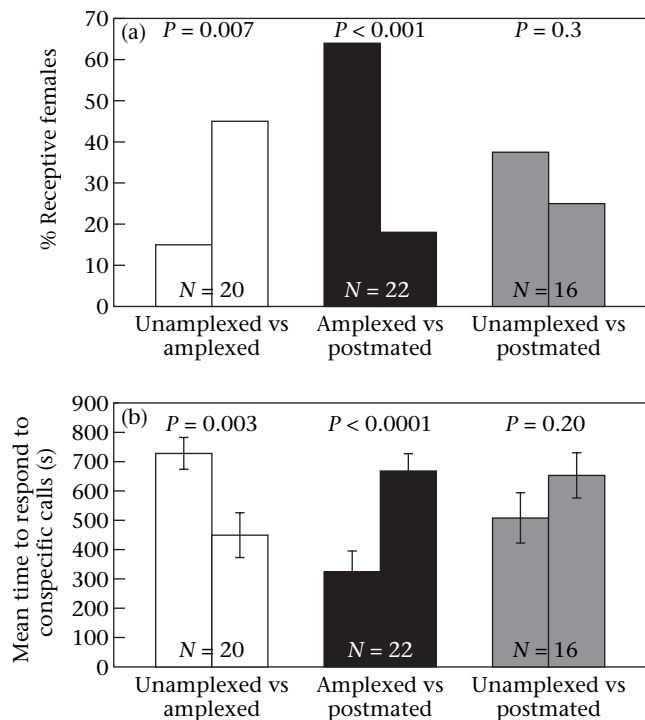


Figure 2. (a) Pairwise comparisons examining whether a female's probability of expressing receptivity to conspecific mate signals varies within reproductive cycles. Each pairwise comparison included only females that completed phonotaxis tests in both reproductive stages. (b) Pairwise comparisons examining whether a female's latency to respond (receptivity) to conspecific mate signals varies within reproductive cycles. Values are means \pm SE.

between the two stages. Six females responded in both conspecific call tests while in the unamplexed stage but once those same females mated, four were responsive to conspecific calls ($N = 16$; $\chi^2_1 = 1.07$, $P = 0.30$; Fig. 2a).

Receptivity of Individual Females

Of the three females that were receptive during the unamplexed stage, two were still receptive in the amplexed stage ($\chi^2_1 = 0.392$, $P = 0.53$; Table 2), indicating that the increase in receptivity was due to nonreceptive females changing to a receptive state when they reached the amplexed stage. In addition, of the 14 females that were receptive during the amplexed stage, three were still receptive during the postmated stage ($\chi^2_1 = 23.77$, $P < 0.001$; Table 2), indicating that the decrease in receptivity was due to receptive females changing to a non-receptive state when they reached the postmated stage. Finally, of the six females that were receptive during the unamplexed stage, two were still receptive during the postmated stage ($\chi^2_1 = 4.27$, $P = 0.04$; Table 2). These results show that there was a significant decline in the receptive responses of individual females from the unamplexed to the postmated stage; however, there was no significant difference in the proportion of receptive females between these stages. Together, these analyses indicate that the receptive females in the unamplexed stage significantly reduced their receptivity while other females began to express receptivity entering the postmated stage.

Further examination of receptivity in individual females showed that there was a significant decrease in the latency to respond to a conspecific mate signal for females that completed the receptivity phonotaxis test in both the unamplexed and amplexed condition (728.02 ± 242.36 s and 449.55 ± 341.70 s, respectively; $t_{19} = 3.339$, $P = 0.003$; Fig. 2b). The latency to respond to a conspecific mate signal significantly increased for the females that completed the receptivity phonotaxis test in both the amplexed and postmated condition (324.02 ± 333.44 s and 668.20 ± 275.27 s, respectively; $t_{21} = -5.18$, $P < 0.0001$; Fig. 2b). There was no difference in the latency

to respond to a conspecific mate signal for females that completed the receptivity phonotaxis test in both the unamplexed and postmated conditions (507.93 ± 343.95 s and 652.9 ± 309.73 s; $t_{15} = -1.325$, $P = 0.205$; Fig. 2b).

Permissiveness Results

Descriptive statistics for all females that completed a permissiveness phonotaxis test in at least one stage showed that the time to respond to an artificial hybrid call decreased from the unamplexed to the amplexed stage and increased again in the postmated stage. Similarly, the frequency of response to the hybrid call increased from the unamplexed to the amplexed stage and declined again in the postmated stage (Table 1).

Proportion of Permissive Females

Among females that were measured in the unamplexed and amplexed reproductive stages, no female had responded to the artificial hybrid call in the unamplexed stage but when those same females entered the amplexed stage, four responded to the artificial hybrid call ($N = 20$; $\chi^2_1 = 5.0$, $P = 0.025$; Fig. 3a). Ten females responded permissively while in the amplexed stage, which decreased to four after they had mated ($N = 22$; $\chi^2_1 = 6.6$, $P = 0.01$; Fig. 3a). Finally, four females responded to the artificial hybrid call while they were in the unamplexed and postmated stages ($N = 16$; $\chi^2_1 = 0.0$, $P = 1.0$; Fig. 3a).

Permissiveness of Individual Females

Clearly, none of the permissive females in the amplexed stage were the same females that had made permissive mate choices in the previous stage ($\chi^2_1 = 0.0$, $P = 1.0$; Table 2). Therefore, the increase in permissiveness was due to nonpermissive females changing to a permissive state when they reached the amplexed stage. In addition, of the 10 females that were permissive in the amplexed stage, two were still permissive in the postmated stage

Table 2. Receptivity and permissiveness of individual females throughout a single reproductive cycle

| Behaviour/Paired stages | Increased responses | Decreased responses | No response, both stages | Response, both stages | Total | Probability of responding at stage 2 |
|-------------------------|---------------------|---------------------|--------------------------|-----------------------|-------|--------------------------------------|
| Receptivity | | | | | | |
| Unamplexed/amplexed | 7 | 1 | 10 | 2 | 20 | 0.53 |
| Amplexed/postmated | 1 | 11 | 7 | 3 | 22 | <0.001 |
| Unamplexed/postmated | 2 | 4 | 8 | 2 | 16 | 0.04 |
| Permissiveness | | | | | | |
| Unamplexed/amplexed | 4 | 0 | 16 | 0 | 20 | 1.0 |
| Amplexed/postmated | 2 | 8 | 10 | 2 | 22 | <0.001 |
| Unamplexed/postmated | 3 | 3 | 9 | 1 | 16 | 0.08 |

Chi-square goodness of fit was used to test whether individual females significantly decreased responses to conspecific and hybrid calls throughout the reproductive cycle. A significant decline in the probability of receptivity or permissiveness along with a significant increase in the proportion of responders (see Figs 2a and 3a) indicates that individual females increased receptivity and/or permissiveness between reproductive stages.

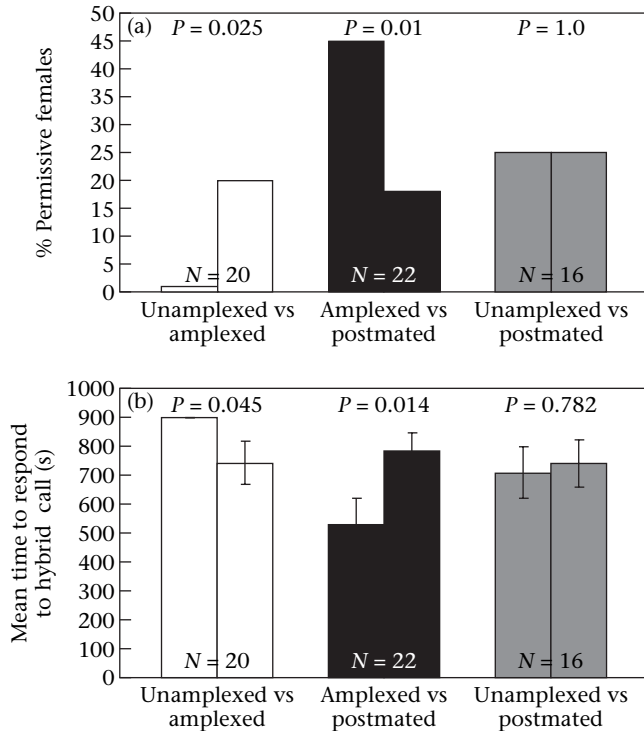


Figure 3. (a) Pairwise comparisons examining whether a female's probability of responding to a less attractive mate signal (permissiveness) varies within reproductive cycles. Each pairwise comparison included only females that completed phonotaxis tests in both reproductive stages. (b) Pairwise comparisons examining whether a female's latency to respond to a less attractive mate signal (permissiveness) varies within reproductive cycles. Values are means \pm SE.

($\chi^2_1 = 11.73$, $P < 0.001$; Table 2), indicating that the decrease in permissiveness was due to permissive females changing to a nonpermissive state when they reached the postmated stage. Finally, of the four females that were permissive during the unamplexed stage, only one was still permissive during the postmated stage ($\chi^2_1 = 3.0$, $P = 0.08$; Table 2). The marginally significant difference between the groups suggests that there was a decline in the permissive responses of individual females from the unamplexed to the postmated stage; however, there was no significant difference in the proportion of permissive females between these stages. Again, these analyses indicate that the permissive females in the unamplexed stage were significantly reducing their permissiveness as females in the postmated stage began to express permissiveness.

Further examination of permissiveness in individual females showed that there was a significant decrease in the latency to respond to an artificial hybrid signal for females that completed the permissiveness phonotaxis test in both the unamplexed and amplexed condition (900 ± 0.0 s and 742.75 ± 327.48 s, respectively; $t_{19} = 2.15$, $P = 0.045$; Fig. 3b). The latency to respond to the artificial hybrid signal significantly increased for the females that completed the permissiveness phonotaxis test in both the amplexed and postmated condition (529.81 ± 415.99 s and 785.23 ± 275.9 s, respectively; $t_{21} = -2.67$, $P = 0.014$;

Fig. 3b). There was no difference in the latency to respond to the artificial hybrid signal for females that completed the permissiveness phonotaxis test in both the unamplexed and postmated conditions (709.0 ± 349.9 s and 739.87 ± 320.5 s; $t_{15} = -0.282$, $P = 0.782$; Fig. 3b).

Discrimination

Females that responded to the whine-chuck in both the first and last phonotaxis tests were considered discriminating. Females that were inconsistent in their responses between these two tests were considered to have non-discriminating responses. No female consistently chose a whine alone in both tests. The results for each reproductive stage are given in Table 3. Nine females responded in both conspecific call tests in the unamplexed stage. Seven of those females consistently chose the whine-chuck. Of the 18 females that responded in both conspecific call tests, 14 consistently chose the whine-chuck in the amplexed stage. In contrast, six females chose the whine-chuck in both conspecific call tests in the postmated stage, but only one female consistently chose the whine-chuck in both tests. The results of a Fisher's exact test revealed that the proportions of discriminatory responses in the unamplexed and amplexed stages were not significantly different ($P = 1.0$). On the contrary, there was a significantly greater proportion of discriminatory responses in the amplexed stage compared with the postmated stage ($P = 0.015$).

We also examined whether the females that responded to the artificial hybrid call were also more likely to show inconsistent responses during the conspecific call tests. Five females behaved permissively in the unamplexed stage (16% permissiveness; $N = 31$). Three of the five showed discriminatory behaviour, one did not, and one did not consistently respond in the discrimination tests. Twelve females behaved permissively in the amplexed stage (35% permissiveness; $N = 34$). Seven of the 12

Table 3. Discrimination throughout a single reproductive cycle

| | Number of discriminatory responses | N | % Discriminatory behaviour |
|------------------------------|------------------------------------|----|----------------------------|
| Frequency of discrimination* | | | |
| Unamplexed stage | 7 | 9 | 78 |
| Amplexed stage | 14 | 18 | 78 |
| Postmated stage | 1 | 6 | 17 |
| Permissive responders only† | | | |
| Unamplexed stage | 3 | 5 | 75 |
| Amplexed stage | 7 | 12 | 70 |
| Postmated stage | 0 | 5 | 0 |

*Probability of expressing discriminatory behaviour in each reproductive stage.

†Number of females that chose the artificial hybrid call in the permissiveness test but consistently responded to the preferred whine-chuck when it was available in the discrimination tests. Only females that responded in both conspecific call tests were considered. No female consistently chose a whine alone.

showed discriminatory behaviour, three did not and two did not consistently respond in the discrimination tests. Finally, five females behaved permissively in the post-mated stage (16.7% permissiveness; $N = 30$). Three of the five females showed no discriminatory response and two did not consistently respond in the discrimination test (Table 3).

Heterogeneity within the Unimplex Stage

All females that oviposited within 24 h from the time they completed their unimplex phonotaxis test ($N = 4$) were receptive to conspecific calls. Only one female out of the 10 that required more time to oviposit were receptive to conspecific calls (Fisher's exact test: $P = 0.005$). In addition, three of the four unimplex females that oviposited immediately after testing responded to the artificial hybrid call, whereas only one female that required more than 24 h to oviposit responded to the artificial hybrid call (Fisher's exact test: $P = 0.04$).

Association between Receptivity and Permissiveness

We rarely observed permissive mate choice in females that did not show receptive behaviour in both conspecific call tests (3.2% of 31 females, 5.9% of 34 females and 6.7% of 30 females in the unimplex, implex and post-mated groups, respectively). Therefore, we examined whether increases in receptivity, as measured by latency to respond to a conspecific signal, were accompanied by an increased likelihood to respond permissively. We divided females according to whether they responded permissively (Fig. 4). Permissive responders required a mean of 196.83 ± 178.33 s ($N = 12$) to respond to a conspecific call, which was significantly less time than the 517.07 ± 355.56 s that nonpermissive responders required to choose a conspecific call ($N = 22$; $t_{32} = 3.49$, $P = 0.001$). Furthermore, linear regression revealed that some of the variation in time to respond to a hybrid call was significantly explained by the female's time to

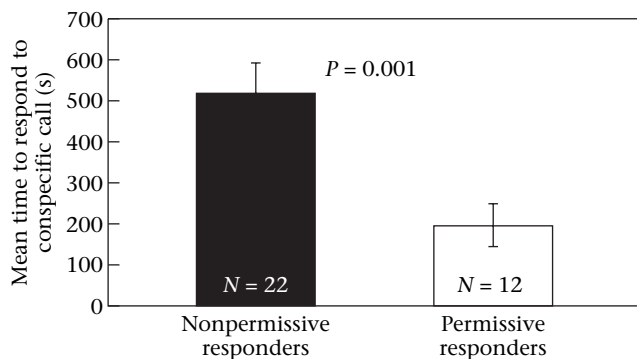


Figure 4. Relationship between a female's motivation to mate (i.e. receptivity) and her permissive mate decisions. Females in the unimplex reproductive stage were divided into permissive and nonpermissive responders and their average time to respond to a conspecific call was compared. Values are means \pm SE.

respond to a conspecific call ($N = 34$; $r^2 = 0.21$; $F_{1,32} = 8.3$, $P = 0.007$).

Latency to Respond and Time to Oviposit

The amount of time that a female requires to respond to any mate signal may be influenced by how much time she has remaining before she must oviposit. Therefore, we examined the relationship between latency to respond in both the receptivity and permissiveness tests and time to oviposit. We divided females according to whether they required more ($N = 11$) or less ($N = 14$) than the median time (14.5 h) to oviposit after their last phonotaxis test. All females that laid eggs within 14.5 h of their last phonotaxis test displayed receptivity, whereas 27% of females that laid eggs more than 14.5 h after their last phonotaxis test displayed receptivity. Females that oviposited within 14.5 h required an average of 86.78 ± 50.38 s to respond to a conspecific advertisement calls, whereas females that oviposited after 14.5 h required 533.7 ± 308.75 s to respond ($t_{23} = -4.7$, $P = 0.001$; Fig. 5). Seventy-one per cent of females that laid eggs within 14.5 h displayed permissive mate choices, whereas 36% of females that laid eggs after 14.5 h displayed permissive mate choices. Females that oviposited within 14.5 h required an average of 310.5 ± 388.74 s to respond to the hybrid call, whereas females that oviposited after 14.5 h required 632.09 ± 380.78 s to respond ($t_{23} = -2.1$, $P = 0.05$; Fig. 5).

DISCUSSION

The results of this study show that two aspects of mate choice behaviour, receptivity and permissiveness, are low during the unimplex stage but increase in the

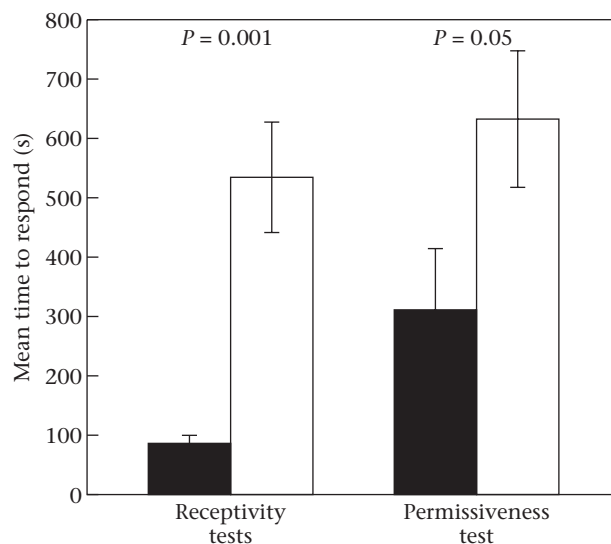


Figure 5. Mean \pm SE time taken by female túngara frogs to respond to a conspecific call and a hybrid call when the time from the last phonotaxis test to oviposition was less than (■) or greater than (□) the median time of 14.5 h.

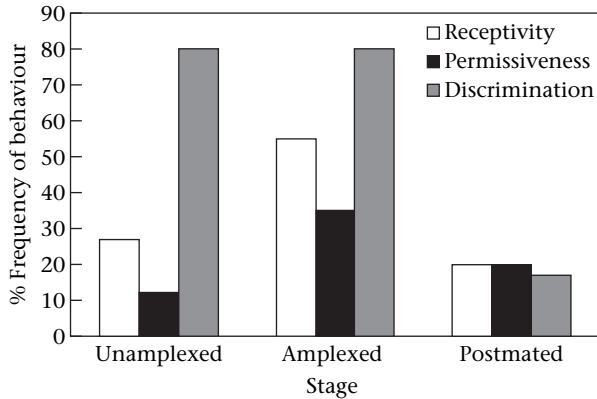


Figure 6. The overall pattern of receptivity, permissiveness and discrimination of female túngara frogs observed in the three reproductive stages.

amplexed stage and decrease again in the postmated stage (Fig. 6). This pattern was shown both in the proportion of females in different reproductive stages expressing receptive and permissive behaviour and in the behavioural changes of individual females moving from the unamplexed to the amplexed state. Furthermore, the mean time to respond to both a conspecific call (receptivity test) and an artificial hybrid call (permissiveness test) significantly declined from the unamplexed to the amplexed stage, then significantly increased again between the amplexed and postmated stages. Overall, these analyses show that the proportions of receptive and permissive females, the receptive and permissive states of individual females and the time to respond to mate signals all change throughout a single reproductive cycle.

If amplexus is considered as a behavioural indicator that females are near the point of releasing eggs, these data suggest that female anurans increase receptivity and permissiveness as time to oviposit approaches. That is, the probability that a female will display any phonotaxis behaviour, the probability that she will display phonotaxis behaviour towards a less attractive mate signal (i.e. artificial hybrid call), and the speed at which she will respond to either signal all increase as time to oviposit approaches. This increase in permissive mate choice, however, is not a result of a decrease in female discrimination. Females retain their ability to discriminate between whines and whine-chucks but are willing to accept a normally unattractive hybrid call when it is the only signal available. Furthermore, our results show that both female receptivity and female permissiveness are correlated and simultaneously increase as females approach the time at which their eggs are ready to be released. This conclusion was further supported by results showing that females that required less time to respond to an attractive conspecific mate call also required significantly less time to respond to the artificial hybrid call compared with females that required more time to respond to a conspecific call. This suggests that as a female becomes more motivated to mate, as measured by her latency to respond to a conspecific call, she will also become more likely to respond permissively. Additional analyses also support the conclusion that both

female receptivity and permissiveness increase near the point of oviposition. First, within the group of unamplexed females, those that oviposited within 24 h of capture were more likely to be receptive and to respond permissively. Second, females that oviposited within 14.5 h of their last phonotaxis test showed higher receptivity than those that took longer than 14.5 h to release their eggs.

Ryan et al. (2003) points out that when female túngara frogs are tested in two-choice phonotaxis tests, they will show strong discrimination in favour of a conspecific call over a heterospecific call; however, when tested in one-choice phonotaxis tests, females will often display some degree of recognition towards the heterospecific call. This suggests that a female will always prefer the conspecific call to the hybrid call in a two-choice test. Therefore, we examined the females' responses to our hybridized (heterospecific/conspecific) call in a single choice test, and we measured the females' overall discrimination in a two-choice test. This type of design allowed us to determine the point at which females begin to accept the hybrid call and whether there is an overall breakdown in female discrimination. The results of our study show that if females do respond to less attractive signals in a single choice phonotaxis test, it does not indicate a lack of discriminatory response, because when we tested the same females in a two-choice phonotaxis test, they still displayed phonotaxis towards the preferred conspecific call. Thus, our results suggest, instead, that the increase in permissive mate choices during the amplexed stage is probably explained by females lowering their threshold for accepting calls, thereby broadening the range of mate signals that were acceptable rather than losing an ability to discriminate among signals. Murphy & Gerhardt (1996) reported a similar result in a study conducted with female barking treefrogs, *Hyla gratiosa*. They tested females with calls that differed either in fundamental frequency or in repetition rate and they found that discrimination of females tested before and after they entered amplexus did not differ.

Interestingly, females in the postmated stage continued to display phonotaxis behaviour despite having released their eggs. There is no clear adaptive explanation as to why females should continue to be receptive to male mate signals when they no longer have eggs to fertilize. The behaviour may instead be the residual effects of whatever mechanism increased female receptivity as time to oviposit approached. For example, it may indicate the gradual clearing of some endocrine product that peaked near oviposition.

During the postmated stage, both receptivity and permissiveness fell to levels seen in the preamplexus stage, again indicating some link in these two processes. Females in this stage, however, were the only ones that did not discriminate between the whine and the whine-chuck. This result is consistent with Lea et al.'s (2000) finding for midwife toads, *Alytes muletensis*, that females show positive phonotaxis after mating but do not reliably discriminate between calls with low (1.5 kHz) and mean (1.8 kHz) frequencies. We do not know whether the lack of consistent preference in our study was because females were unable to

distinguish between the two test stimuli or because females perceive the differences as unimportant; that is, whether the differences in the test stimuli were 'just noticeably different' or 'just meaningfully different' (Nelson & Marler 1990). Nevertheless, the results from the postmated stage support the conclusion that changes in permissiveness are not strictly tied to changes in the female's ability or willingness to discriminate between signals.

Both an increase in receptivity and permissiveness may increase a female's probability of having her eggs fertilized, thereby increasing the probability that the high cost of creating and maintaining her oocytes will not be wasted. Current theoretical models of mate choice behaviour make opposing predictions about the direction of change in female choosiness under time constraints. Real (1990) presented a model predicting that the criteria determining the acceptance of a potential mate should be relaxed if mate choice occurs under time constraints. Moore & Moore (2001) recently provided empirical support for this model by showing that time constraints due to reproductive ageing cause female cockroaches, *Nauphoeta cinerea*, to reduce choosiness during mate choice tests. Alternatively, however, Crowley et al. (1991) presented a model predicting that females under time constraints caused by the approach of the end of the breeding season should increase choosiness. This model makes these predictions for animals that have only a single breeding season during which they repeatedly reproduce. Qvarnström et al. (2000) recently provided support for this model by showing that female collared flycatchers, *Ficedula albicollis*, display flexibility in their preference for the size of male forehead patch over the course of the breeding season. Their results showed that female preference becomes more marked, rather than less marked, as time becomes limited near the conclusion of the season. They also reported that large-patched males adjust their behaviour to allocate more effort into postmating reproductive activities later in the season and as a consequence, females that mate with them late in the season benefit through increased reproductive success. In addition, Forsgren (1997) showed that female sand gobies, *Pomatoschistus minutus*, become more selective later in the breeding season. The opposing predictions of theoretical models as well as evidence provided by empirical studies indicate that life-history traits of an animal, such as the number of breeding seasons the adults will experience and/or whether the adults provide parental investment to the offspring, should be considered when making predictions about the directional change of female choosiness. However, regardless of whether female choosiness is relaxed or intensified under time constraints, the significant result in these mate choice studies is that females do show flexibility in their mate decisions over time, indicating that mate choice is in fact not static.

The results of our study indicate that reproductive stage can influence the expression of three aspects of female mate choice behaviour: receptivity, permissiveness and discrimination. Furthermore, it appears that receptivity and permissiveness may be associated so that changes in one behaviour are paralleled by changes in the other behaviour. One explanation for this result is that these

behaviours may be influenced by a common mechanism. Because these behaviours appear to vary according to reproductive stage, it is possible that they are under the influence of the same neuroendocrine mechanisms. The neuroendocrine mechanisms that contribute to female receptivity are well understood in a variety of taxa including birds (Noble 1973; Delville & Balthazart 1987) amphibians (Diakow & Nemiroff 1981; Schmidt 1984, Schmidt, 1985; Boyd 1994) and mammals (Tetel et al. 1994; Cushing & Carter 1999). One mechanism by which hormones could modulate permissive behaviour is by direct action on sensory neurons. For instance, Yovanof & Feng (1983) found that intraventricular injections of oestradiol increase average midbrain auditory-evoked responses in *Rana pipiens*. Additionally, Bereiter & Barker (1980) showed that injections of oestradiol cause receptive field areas of trigeminal mechanoreceptive neurons to enlarge in female rats. Furthermore, these authors found that receptive field area varied significantly throughout the oestrous cycle, with the largest area appearing during oestrus and the smallest area appearing in dioestrus.

The results of our study provide support for theoretical models that predict that female mate choice should be plastic if it occurs under time constraints and indicate that changes can occur on a timescale as short as a single reproductive cycle. We found that, as the time to deposit eggs approached, females increased receptive and permissive mate choice behaviours. Furthermore, these two behaviours fluctuated in parallel throughout the breeding cycle. We also report that the increase in permissive mate choices was not due to a decrease in discriminatory responses but rather indicates a change in a female's threshold for accepting unattractive calls. It is possible that receptivity and permissiveness share a common mechanism so that changes in one behaviour are accompanied by changes in the other behaviour. Future studies may reveal whether a common mechanism can influence these behaviours.

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