fect the balance between inhibitory and excitatory effects of serotonin on behavior. The present model might be useful for testing such interactions.

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9. Cannulas were made from 23-gauge hypodermic needles turned down in a metal lathe to a hub 8 mm long and 4 mm wide, and were threaded inside to accept inner or infusion cannulas. Inner and infusion cannulas were made from 30-gauge hypodermic needles turned down and threaded to fit into the outer cannulas so that their tips protruded 1.5 mm beyond the outer tips. The cannulas were implanted 1.4 mm lateral to the lambda and 4 mm below the top of the skull in rats anesthetized with chloral hydrate, and were secured with skull screws and dental cement. Intrathecal catheters were made from PE 10 polyethylene tubing, as described by T. L. Yaksh and P. R. Wilson [*J. Pharmacol. Exp. Ther.* **208**, 446 (1979)].
10. In intact animals, excitatory motor effects caused by serotonin or its precursors occur only in the presence of a monoamine oxidase inhibitor. Therefore the rats were first treated with a moderate dose of pargyline, which preliminary studies indicated does not alter baseline startle levels. In order to equate conditions for both lateral ventricle and spinal placements, pargyline was given to all animals. After completing the dose-response curves, we found, however, that higher amounts of serotonin (200 to 400 μg) heightened the startle response when given intrathecally without pargyline pretreatment. Since Geyer *et al.* (4) already showed that serotonin given alone intraventricularly depresses startle, pretreatment with a monoamine oxidase inhibitor is not required to demonstrate forebrain versus spinal effects of serotonin on startle.
11. The apparatus used to measure startle is described by G. T. Weiss and M. Davis [*Pharmacol. Biochem. Behav.* **4**, 713 (1976)]. Briefly, five separate stabilimeters were used to record the amplitude of the startle response. Each stabilimeter consisted of an 8 by 15 by 15 cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame. Cage movement caused displacement of an accelerometer; the resultant voltage was proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage during the first 200 msec after the stimulus and was measured with a sample-and-hold circuit. The stabilimeters were housed in a dimly lit, ventilated, sound-attenuated chamber 1.1 m

from a high-frequency speaker. The startle stimulus was a 90-msec, 115-dB burst of white noise with a rise-decay time of 5 msec. Background white noise, provided by a white noise generator, was 46 dB. Sound level measurements were made in the cages with a General Radio model 1551-C sound level meter (A scale).
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Female Mate Choice in a Neotropical Frog

Abstract. *Female* Physalaemus pustulosus *choose their mates and are more likely to choose larger males. There is a significant negative correlation between the size of the male and the fundamental frequency of one of the components of its advertisement call. Playback experiments demonstrate that females are capable of choosing larger males by distinguishing among differences in spectral components of the advertisement call.*

Darwin (1) proposed that sexual selection has two principal components: competition among males (intrasexual selection) and choice by females (intersexual selection). The role of male-male competition has been demonstrated (2); however, the importance of choice by females, especially choice based on male traits, has been disputed since Darwin first proposed the theory (3). Studies have shown that mate choice by females is based at least in part on resources controlled by males (4), and on male position on a lek (5), or rank in a social hierarchy (6). Attempts to show that females in natural populations prefer certain male morphological or behavioral characteristics have been frustrated by difficulties in separating the effect of these characteristics from that of the other factors influencing mate choice (7). Only laboratory studies of *Drosophila* have adequately demonstrated female choice based on male traits (8). I report that female choice of larger males influences male mating success in a neotropical frog, and that this choice is based on one male phenotypic characteristic, the fundamental frequency of an advertisement call component, which is correlated with size and probably age.

The breeding behavior of *Physalaemus pustulosus* (Leptodactylidae) was monitored in a small cement pool on Barro Colorado Island, Panama, for 12 weeks from June to August 1978. Individuals were captured, measured (snout to vent), and given a toe clip for per-

manent identification. Numbered pieces of surveyors' flagging were stitched to the middorsal surface, allowing undisturbed identification during behavioral observations.

Breeding in *P. pustulosus* occurs throughout the year, but is concentrated during the wet season (April to December). As with most anurans that have a prolonged breeding season, the sex ratio at the breeding site was skewed toward males (9). Males advertised from calling sites, and the females approached and initiated amplexus. Usually, a female was present in the pool only on the night she mated. The females seemed to choose their mates freely, although on several occasions noncalling males intercepted females that were en route to calling males. The males often fought each other, but in over 500 hours of observations during 1978 and 1000 hours in 1979, I never saw an unmated male displace a male in amplexus. Males constructed foam nests during amplexus by beating the jelly matrix of the egg mass with their hind legs as they fertilized the eggs (10). Nest building usually occurred 1 to 4 hours after the beginning of amplexus. The males were not territorial and did not defend resources. There was no relation between a male's calling site and the site used for oviposition or his ability to attract mates.

In 1978, I marked 185 males and observed 103 matings. As Fig. 1 shows, the larger males were more likely to acquire mates. However, only some of the males

were present on a given night. Therefore, I examined the effect of male size on mating success for each night during the study. The size of each successful male was compared to the mean size of males present on that night. Males larger than the mean size were more likely to acquire mates ($P < .0001$, one-tailed sign test).

The advertisement call of *P. pustulosus* has two components, the whine and the chuck (*11*). The whine is always present and is followed by zero to six chucks (Fig. 2). Males increase the complexity of the call by adding chucks, and females prefer the more complex call (*11*). I attempted to determine whether the chuck contains information about male size that females might utilize in choosing mates. The fundamental frequency of anuran vocalizations is determined largely by the passive components of the vocal apparatus, especially vocal cord mass (*12*). A negative correlation between size and the fundamental frequency of the advertisement call has been demonstrated for several anuran species (*13, 14*).

The fundamental frequency of the chuck component of the calls of 136 males was determined during 1978 and 1979 (*15*). There was a significant nega-

tive correlation between male size (26.0 to 33.5 mm) and fundamental frequency (200 to 270 Hz, $r = -.53$, $P < .01$) (Fig. 3). The size range of these individuals was similar to that of males observed in the field (25.0 to 34.0 mm). The results indicate that the chuck component does contain information that might allow females to discriminate in favor of larger males. The coefficient of determination ($r^2 = .28$) indicates a low predictability for the size-frequency correlation and suggests that this information might be reliable only for discrimination among males of large size differences.

During 1979, I tested the hypothesis that females are capable of choosing between males at extremes of the size range on the basis of differences in the fundamental frequency of the chuck component. Advertisement calls were synthesized that contained identical whine components but that had chucks of different fundamental frequencies (Fig. 2). The testing apparatus consisted of an octagonal arena 150 cm in diameter, with a burlap wall around the perimeter and two speakers opposite each other and outside the wall. A mirror was placed above the arena at an angle that permitted observations out of direct sight of the frog. The arena was marked into eight sections, 50 cm along each edge of the perimeter. A "speaker response" was noted if the female contacted the burlap wall in a section adjacent to a speaker (within 12 cm) (*16*).

Eight females were presented calls containing chucks with fundamental frequencies of 200 and 260 Hz and with an intensity of 70-dB sound pressure level (SPL) in the center of the arena. Each female was tested once. All eight responded to the speaker producing the call with the lower fundamental frequency ($P = .004$, one-tailed binomial probability). This indicates that females can discriminate among males on the basis of spectral differences in the advertisement call. Reinterpretation of earlier studies of spectral components used in species recognition suggests that intraspecific mate choice based on small frequency differences might be a widespread phenomenon in anurans (*17*).

Sexual selection theory predicts that female choice is based on material or genetic benefits provided by the male (*18, 19*). I have no evidence that females receive different material benefits (such as differences in nest quality) from males, although this possibility is being investigated.

Growth of amphibians is indeterminate; in general, larger males are older. There might be some inconsistencies
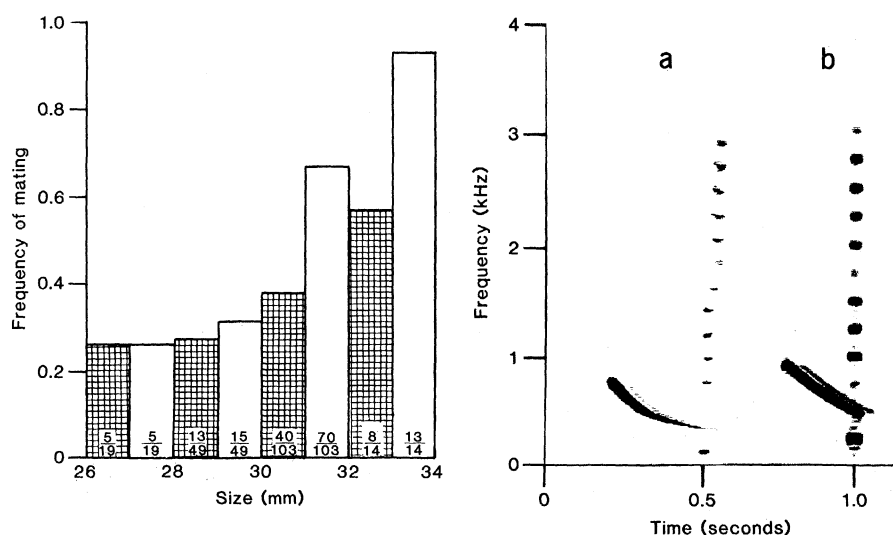

Fig. 1 (left). Differential mating success of male *P. pustulosus*. Hatched bars represent the frequency of successful males (at least one mating) in each male size class; the numerator is the number of successful males and the denominator is the total number of males. Open bars show the frequency of total matings for each size class; the numerator is the number of matings and the denominator is the number of males.   Fig. 2 (right). Natural and synthesized advertisement calls of *P. pustulosus*. (a) Sonogram of a natural advertisement call, a whine followed by a chuck; (b) sonogram of a synthesized advertisement call. All synthesized calls had the same whine component, but the fundamental frequency of the chuck was varied.
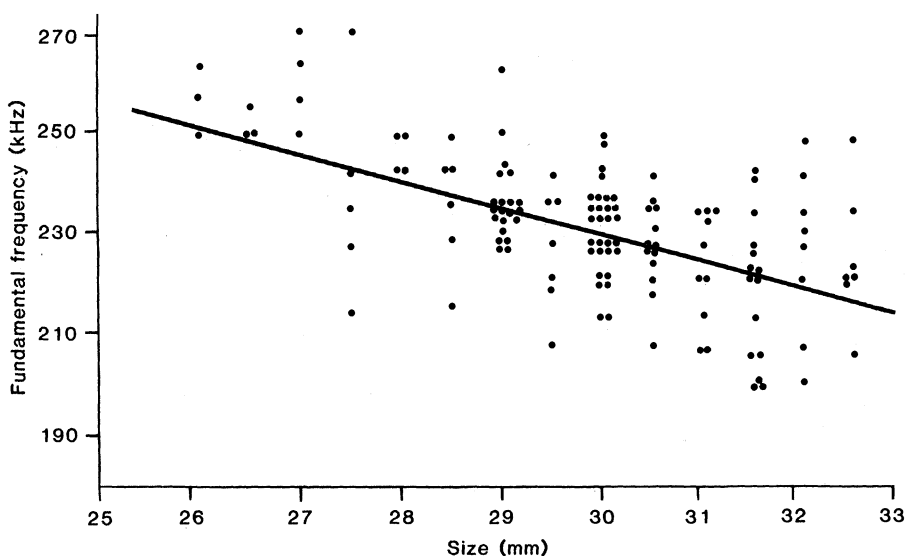

Fig. 3. Fundamental frequency of the chuck component of the advertisement call plotted against male size (snout to vent) for 136 *P. pustulosus*.

in the size-age correlation among males due to differences in resource accrual ability (20). By choosing larger males, females might be selecting mates who have demonstrated the adaptiveness of their genomes for survival and resource accrual ability, thus transferring this benefit to their offspring. Borgia and West-Eberhard (19) have disputed many of the arguments suggesting that the genetic variance in fitness is rapidly depleted by female choice based on male genetic qualities.

I am not suggesting that male-male competition plays no role in the mating system. Males might enhance their mate attraction ability by inhibiting other males from calling or by being present at the breeding site on nights of increased female activity. However, I have provided an experimental paradigm to demonstrate the role of female choice as a determinant of male mating success in a natural anuran population.

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15. Advertisement calls were recorded with a Sennheiser microphone on a Nagra IV tape recorder at a speed of 9.525 cm/sec. Calls were analyzed on a Kay sonograph with narrow bandwidth and a range of 40 to 4000 Hz. The fundamental frequency of the chuck was determined by measuring the frequency distance between the first three visible harmonics on the sonogram and dividing by three (Fig. 2).
16. Playback experiments were conducted in a partially screened room; temperature during the experiments was the same as that outdoors. Sound intensity was equalized at 70 dB SPL in the center of the arena. Sound intensity measured halfway between the speaker and the arena center was that predicted by the inverse-square law (76 dB SPL). Sound intensity of each speaker was measured at several places in the arena; at corresponding sites, intensity was within 1 dB SPL for each speaker. Speakers were 150 cm apart; frogs at the release site were within one wavelength of sounds less than 440 Hz and thus were subject to near-field energy from some of the chuck harmonics. In the field, however, females might approach to within several centimeters of a calling male before retreating and proceeding to other calling males. The roles of near- and far-field energy in the neurophysiological mechanism of female choice are not known. Calls were broadcast by two speakers, each driven by a Nagra IV tape recorder, and were switched between speakers after each test and between tape recorders each night. The call presented first was determined by the flip of a coin. A female was placed in the center of the arena in a wire cylinder. Calls were emitted alternately from the speakers every 1 second. The cylinder was removed 30 seconds after the calls began, and the female's behavior was recorded. "No response" was recorded if the female did not contact the wall before the tape ended (3.5 to 4.0 minutes).
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