Mate preference in female electric fish, *Brachyhypopomus pinnicaudatus*

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Weakly electric fish communicate with brief electrostatic field pulses called electric organ discharges (EODs). EOD waveforms are sexually dimorphic in most genera, a condition thought to result from mate choice acting to shape the electric signal's constituent action potentials. We have no direct behavioural evidence that sexual selection by either mate choice or intrasexual competition is responsible for sex differences in the EOD waveforms of electric fish. We explored sexual selection in electric fish by conducting two-choice unforced preference tests with live, unaltered gymnotiform electric fish, *Brachyhypopomus pinnicaudatus*, which are sexually dimorphic. In the initial test, gravid females selected males over females only when the males were larger than average. Gravid females in later tests preferred larger males to smaller males in a significant majority of those trials in which they showed a preference. In about one-third of those trials, females spawned with their preferred male, confirming their preference. We concluded that passage through the choice apparatus was related to mate choice. The signals of chosen males had larger EOD amplitudes and longer EOD durations. These findings show that female *B. pinnicaudatus* do have a preference for a certain male phenotype. The system requires additional study to dissociate correlated male phenotypic characters to identify which male traits the female prefers.

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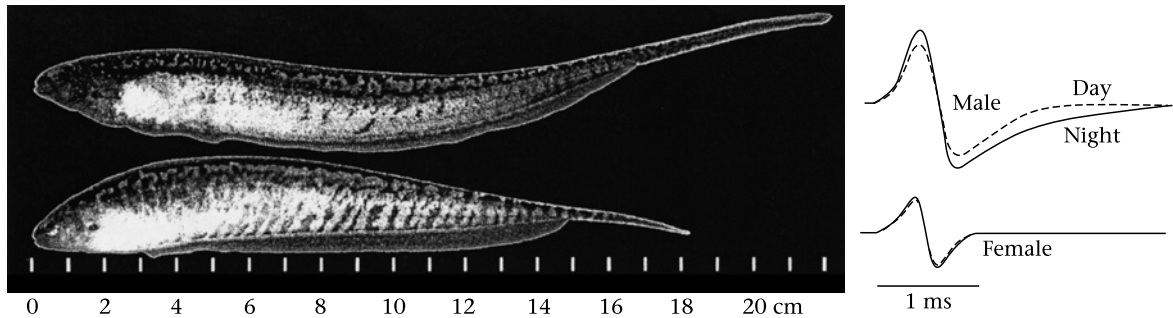
Neuroethological studies of electric fish have given us rare insight into the intimate ties between the neural pathways of the sensory and motor systems of a vertebrate animal (Heiligenberg 1991). Electric fish could equally well enhance our understanding of how sexual selection modifies the neural processes that regulate courtship and reproductive behaviour. Many electric fish show pronounced sexual dimorphism in their electric signals and their electricity-producing structures (reviewed in Hopkins 1999), strongly implicating sexual selection as a key driving force in the evolution of electric communication. Mate choice in particular has been proposed to underlie sexual dimorphism in the electric signal (Hopkins et al. 1990). That electric communication signals are sexually dimorphic is not, by itself, a reliable indicator that female choice has shaped the communication system. Sexually dimorphic signal traits may function solely in competition between males vying to control resources the females need to reproduce (Searcy & Andersson 1986; Hopkins et al. 1990).

Studies of communication between electric fish of the New World order Gymnotiformes have shown that females distinguish between EODs (electric organ dis-

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charges) of males and females (Kramer & Otto 1988; Shumway & Zelick 1988; Kramer 1999) but not that females discriminate between individual male EODs or even that male EODs are attractive to females. We do not know whether female gymnotiform electric fish show preferences based on male phenotype that would lead to active mate choice. In the present study, we hypothesized that a female would show mate preferences based on male EOD, body size, or both.

We studied pintail knifefish of the genus *Brachyhypopomus* (order Gymnotiformes, family Hypopomidae), a group known for extreme sexual dimorphism in their signals and electric organs (reviewed in Stoddard et al. 1999). The EOD of *B. pinnicaudatus* is a biphasic waveform, the second phase of which is much longer in duration in males than females (Fig 1). Hypopomids constantly emit their pulsed EODs for electrolocation purposes, and modulate their discharge rates to produce signals of courtship and aggression (Westby 1975; Hagedorn 1988; Kawasaki & Heiligenberg 1989). In *B. pinnicaudatus* (Hopkins 1991), the species for which our observations are most complete, courtship begins only after a female has approached a particular male (Stoddard et al. 1996). Thus, it appears the male's EOD waveform or body size could be used in the female approach decision.

Figure 1. Male and female *B. pinnicaudatus* shown with their respective electric organ discharge (EOD) waveforms. The male's EOD has both a larger amplitude and a greater duration in the second phase. Both of these waveform characters are further enhanced at night when the fish are active. The female's EOD changes little between day and night.

Before initiating detailed studies of the neural basis of mate choice in electric fish, we must determine whether female electric fish actually show a preference for a male phenotype that leads to a mate choice, as opposed to choosing an oviposition site defended by a particular male. The next step would be to determine whether preference is based on sexually dimorphic properties of the male's signal (Huxley 1938). Ultimately, we would want to know whether female preferences persisted under natural conditions that included the full suite of potential interactions, including predators, competition between males and variable resources (Searcy & Andersson 1986).

Our goal in this study was to address the first issue: do female electric fish show a preference between prospective mates and do these preferences lead to the choice of a mate? We allowed females to chose between two prospective mates, each behind a plastic screen barrier that allowed the female to sense the signals of the choice fish but that prevented direct contact or any assessment of oviposition sites, which were identical. Fish were active only in total darkness; thus, assessment of and choice between prospective mates, indicated by passage through a one-way funnel net in the barrier, would be based on characters such as electric or chemical signals, or possibly water vibrations. If the female passed through a funnel, she had the option of spawning with that male. Significant patterns in passage through the trap doors would indicate a preference for particular classes of fish (e.g. males of a particular size). If females then spawned with the males they preferred, male taxis could be viewed as an indication of active mate choice. The apparatus creates an artificial situation in which a female may get stuck with a male while merely inspecting him at close range. This disadvantage was offset by the ability to keep the choice fish apart so that they did not suppress one another's behaviour through direct aggressive interaction. Furthermore, the females could and did interact electrically with the males during the day and at night before passing through the funnel.

METHODS

Holding Conditions

We identified 60 male and 33 female seventh- and eighth-generation *B. pinnicaudatus*, descended from a small founding population, and separated them from an outdoor captive-breeding colony. We conducted experiments during 28 April–7 December 1998. In the summer months, experimental fish resided outdoors in 450-litre pools ($185 \times 95 \times 26$ cm). When daylight fell below 14 h, the fish were moved indoors into 700-litre aquaria ($180 \times 62 \times 65$ cm) where light cycles could be controlled. Pools and aquaria were filled with deionized water that was adjusted and maintained at a conductivity reading of $100 \pm 30 \mu S/cm$. Natural south Florida sun heated the pools and shade was provided by water hyacinths, *Eichhornia crassipes*, which covered the surface and provided shelter for the fish. We isolated 11 females per pool without males present and held males in separate pools of 20 fish. All fish were fed oligochaete blackworms ad libitum to encourage egg production in the females and courtship behaviour in the males. We simulated breeding conditions indoors with artificial rain (recirculated water falling on the surface), 14 h of light, water conductivity of $80 \pm 20 \mu S/cm$, $27 \pm 2°C$ water temperature and blackworms. All females used in tests came from the same group of 33 females.

Testing Conditions

The testing tanks ($180 \times 62 \times 65$ cm, 700 litres) were divided into three equal sections with electrically transparent screening (Fig. 2). A water hyacinth and a 30-cm long PVC pipe were placed in each section for refuge. The water hyacinth also served as a potential site for oviposition. A plastic mesh funnel cone (Fig. 2) facilitated one-way passage of the female from the centre compartment to either side compartment where her choices resided. A semistiff net door kept the funnel closed, and the female had to push hard to open it and pass through. After her passage, the net door closed again. Therefore, neither she nor the choice fish on the narrow side of the funnel was able to return. Once a female made her choice, she was trapped there until we removed her. The funnel cones could also be blocked shut with a rubber stopper to prevent passage.

Unforced Two-choice Tests

We conducted three tests; each consisting of a series of unforced preference trials on gravid females (Table 1).
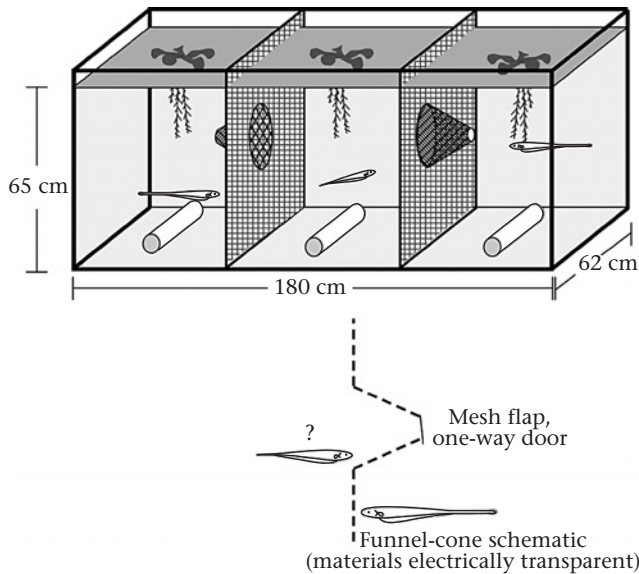
Figure 2. The test female is shown in the centre of the testing tank with potential mates on either side. She may pass through the funnel cones to join a male on one side or the other. Plastic screening that allows electrical and chemical contact but not physical contact separates the fish. The room is completely dark at night and the fish remain hidden until darkness so that they cannot see one another. The funnel cone can be locked closed or opened to allow the test female one-way passage into a compartment with the fish of her choosing.

Test 1, in which females chose between mature males and females, was designed to determine whether gravid females (N=28) showed meaningful mate preferences in our apparatus. We expected to use the results from this test as a predictor upon which to base subsequent tests. Test 2 was designed to determine whether gravid females preferred large males to small males. We gave 33 females a single night to select between two males differing in length and thus EOD duration or amplitude (Fig. 5, Table A1, Appendix). In test 3, we gave 31 females up to three nights to make a choice. Our intent here was to decrease nonmovements and thus increase the proportion of apparent choices that would lead to spawning.

Males in each pool were distinguishable by size, and we assigned a number to each male presented to a female to ensure that no female was run twice with the same male. Males and females offered to the test females for choice were assigned randomly to either left or right tank compartments to eliminate potential side bias. Males were used once in each trial in series 1 and 2 and never presented to the same female more than once. However, in the third series, we used 12 males a second time, but only after an interval of several weeks. Six of the 31 females in test 3 were tested more than once, either because of equipment malfunction in their first trial, or because they did not choose in their first trial. However, two of these six never chose and one died before choosing. The females were run in all three tests after rest periods ranging from weeks to months. Female *B. pinnicaudatus* are serial spawners. Gravid females taken from the colony were not necessarily virgins, so we made no attempt to control for mating experience either before or between tests.

We analysed preference based on choice data using a binomial test with the one-tailed prediction that females would choose males over females and larger males over smaller ones. We excluded from the analysis trials in which the female did not move to either side.

Test 1

We placed one female in the centre chamber of the test tank, a male in one end chamber and a female in the other end chamber. The test nights began with the funnel cones blocked to prevent choice artefacts from random exploration. Beginning 1–2 h after lights out, we monitored electric signals in the tanks using silver dipole electrodes positioned on each electrically transparent barrier. The electric signals from multiple tanks were multiplexed, tape-recorded and monitored on an oscilloscope and audio speakers. If we detected courtship 'chirps' (i.e. brief high-frequency, low-amplitude signal discharges; Hagedorn 1988; Kawasaki & Heiligenberg 1988; Stoddard 2002), we unblocked both funnel cones on the dividers. The females were then left overnight to choose. Passage to either side compartment was considered the female's display of preference (or possible rejection of the other fish), and nonmovement was taken as disinterest. The next morning, we noted all movements and checked the plants for eggs.

Table 1. Overview of the experimental designs of the three tests for mate preference

Test	N	Choice	Nights	Test period
1	28	Male:Female 23.6±1.92 cm:N/A	1	28 April–27 May 1998
2	33	Large male:Small male 23.8±0.71 cm:16.8±1.20 cm	1	28 May–22 June 1998
3	31	Large male:Small male 24.5±1.91 cm:18.0±1.91 cm	1–3	23 June–7 December 1998

Mean±SD of total body length is shown for choice males in each test. Test 1, in which females selected between mature males and females evaluated whether gravid females showed a meaningful indication of preference in our apparatus. Test 2 evaluated whether gravid females preferred large to small mature males. Test 3 provided females up to three nights to make a selection, which increased the number of selections made and resulted in significant levels of spawning.

Test 2

One female was placed in the centre chamber of the test tank and a male was placed in each end chamber ($N=2$). The males were measured from snout to tail, and each pair differed in length by at least 4 cm. The funnel cones were unblocked after lights out, and the females left overnight to choose (or not) without monitoring for male courtship signals.

Test 3

Conditions were the same as test 2, except that females that did not move on the first night were given two more nights to show a preference. We recorded male characteristics in this test for later comparison with female preferences. Each male was weighed and measured from snout to tail before testing. We also digitized the males' EODs on both the evening of the test and the day after. We placed each male to be digitized in a soft mesh hammock suspended in the centre of the recording tank ($122 \times 56 \times 46$ cm, 300 litres). The large size of the measurement tank was necessary to eliminate boundary effects that affect the EOD waveform. Carbon rod recording electrodes were positioned 122 cm apart at either end of the tank, with an Ag/AgCl wire midtank connected to the building ground. The electrodes were connected to an AC-coupled differential amplifier (Charles Ward Electronics BMA-200) with high-pass filter at 1 Hz, low-pass filter at 10 kHz, and gain of $100 \times$ or $200 \times$. We digitized EODs at 125 kHz with 16-bit resolution (Tucker Davis Technologies System 1 AD2). Body flexion, rostrocaudal position and tank boundary effects are the largest sources of measurement error. Careful calibration has shown that this system (tank size, positioning system, water conductivity and electrode geometry) allows ± 10 cm of rostrocaudal motion with no measurable alteration of EOD amplitude or waveform (Franchina & Stoddard 1998). A less controllable source of error is that the EOD waveform of male B. pinnicaudatus varies on a circadian rhythm, peaking around the hours of spawning when the fish were otherwise engaged in the experiment (Franchina & Stoddard 1998). EODs are also affected by the social situation (Franchina et al. 2001). Because we were unable to record calibrated EODs during the choice experiment, we measured EOD values of males in the late afternoons before and after testing and averaged these values for each male. We measured EOD amplitude as the peak-to-peak values of the digitized waveform and the duration of each phase at 10% of the total peak-to-peak amplitude, then summed them for total duration (Fig. 3).

RESULTS

Test 1

We spent numerous nights of monitoring without detecting 'chirps', probably because females were just coming into breeding condition for the season. Beginning 28 April 1998, we detected the male courtship
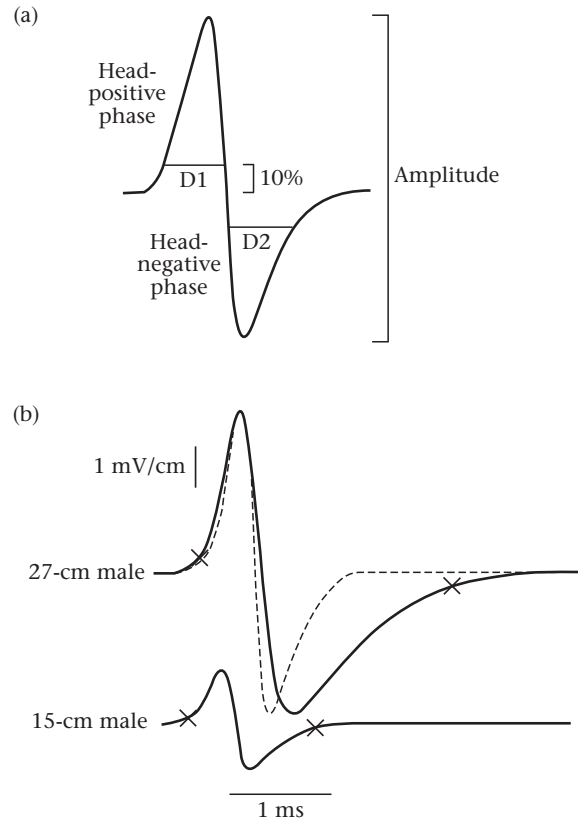

Figure 3. (a) EOD amplitudes were measured peak-to-peak. EOD phase durations (D1 and D2) were measured at 10% of peak amplitude then summed for total duration. (b) EOD waveforms of two males recorded head-to-tail with electrodes 122 cm apart: a very large 27-cm male and a very small 15-cm male. The thin, dashed line is the EOD of the small male, scaled in amplitude to compare its duration characteristics with the EOD of the large male. The Xs in each waveform indicate the 10% amplitude points used to delineate EOD duration.

signals on some evenings, and on these nights we opened the funnel cones, allowing the test female to move. Of the 28 females allowed to move, 22 did so. The differential taxis towards males was not significant: 13 females moved towards males and nine towards females (binomial test: $P=0.26$). Two females (15%) spawned with these males. However, the chosen males were 2.4 cm larger, on average, than the rejected males (one-tailed t test: $t_{20}=3.60$, $P=0.001$; Fig. 4). Therefore, we hypothesized that if females were rejecting unacceptable males when they moved towards a female, then tests 2 and 3 should support our hypothesis of a preference based on size or signal features.

Test 2

Of the 33 females tested, only nine showed a preference. Eight of the nine females that chose sides did move towards the larger male (binomial test: $P=0.02$). Three of the nine females spawned with their preferred males, in every case the larger male. As before, the preferred males
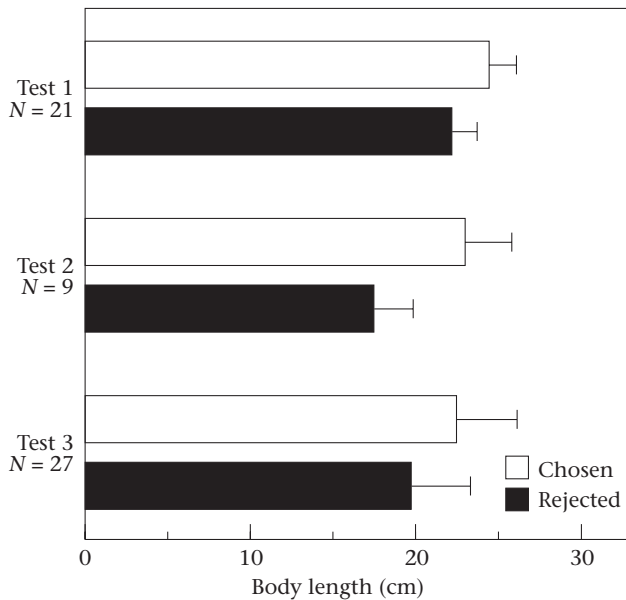
Figure 4. Mean±SD lengths of males selected and rejected by females in the three tests. All differences were significant (P=0.001, 0.004, 0.03, respectively).

were indeed longer than rejected males (one-tailed t test: t_8=3.44, P=0.004; Fig. 4).

Test 3

Testing across multiple nights greatly increased the percentage of females moving towards males. Of 31 females tested, 28 moved and 20 of those preferred the longer male (binomial test: P=0.018). Eleven females spawned with their preferred males, seven with the larger male and four with the smaller. Nineteen females moved towards the male with the longer duration EOD (binomial test: P=0.04) and 19 moved towards the male with the higher amplitude EOD (binomial test: P=0.04). Sixteen moved towards the male with both the longer duration and higher amplitude, and three moved towards the male with either the longer duration or the higher amplitude. In the only case where both the parameters were inversely related to body length, the female moved towards the smaller male with both the larger amplitude and the longer duration. Once again, when the preferred males were compared to the rejected alternatives, they were longer from snout to tail (one-tailed t test: t_{27}=3.44, P=0.03; Fig. 4). Differences in EOD duration and amplitude between selected and rejected males were in the same direction, but P values were 0.06 and 0.15, respectively. Body length, EOD duration and EOD amplitude were all positively correlated in the males used in this study (linear regression: length:duration: r^2=0.19, P<0.001; length:amplitude: r^2=0.58, P<0.001, duration:amplitude: r^2=0.12, P<0.01; Table A1, Appendix, Fig. 5), as found in previous studies of *B. pinnicaudatus* (Hopkins et al. 1990; Franchina & Stoddard 1998). Neither body weight nor weight-to-length ratio indicated that body condition influenced mate preference independently of body length or EOD.

DISCUSSION

Our results show that gravid female *B. pinnicaudatus* did prefer certain males in our captive, nonforced choice tests. Furthermore, these preferences frequently led to mate choice, defined as successful spawning. Under these conditions, preference was based on some aspect of the male phenotype that correlated with male size: longer males were preferred. Body length correlated strongly with EOD amplitude and weakly with EOD duration (Fig. 5). Our data were equally consistent with the selection of any of these traits by the females. Thus, although we are confident that females do have preferences for certain males, we are not yet sure which characters are preferred.

In general, female fish prefer larger males as mates (Ryan & Keddy-Hector 1992), but male body size may be less evident to a female electric fish than some of its correlates. We think it unlikely that females assessed male size visually. The fish emerged from their hiding places only in total darkness, and females were separated from males by dark plastic screens. Ascertaining body size, either through electric sense or lateral line, requires the two fish to engage in parallel swimming, which is difficult although not impossible through the screen barriers. Infrared video studies show that during early stages of courtship, female *B. pinnicaudatus* frequently approach a male's tail where EOD amplitude is greatest, but they do not swim parallel to the male until they begin to spawn (P. K. Stoddard, M. Kilburn & K. Patterson, unpublished data). Preliminary data for males separated similarly indicates that they make assessments using EOD amplitude over body size (V. Salazar, M. Markham & P. Stoddard, unpublished data).

The correlation between EOD amplitude and body length may loosen at higher EOD amplitudes (Fig. 5). Thus, females might have more reliable information to discriminate against small males than between relatively large males, an idea consistent with females' apparent minimum threshold for body length observed in test 1. However, if males with large-amplitude EODs also have larger day–night oscillations, as we suspect, then the EOD data presented here, collected at times bracketing the hours of choice, may be more variable than those of smaller males. These speculations assume that females could actually assess EOD amplitude, a complex task, given that EOD amplitude varies nonlinearly with distance (Stoddard et al. 1999). Using electric sense, mormyrid electric fish can resolve the object size–distance problem (von der Emde et al. 1998), and *Gymnotus* can assess a dipole signal's source location if the fish is familiar with the signal (Scudamore & McGregor 1993). Furthermore, the electric fields of male *B. pinnicaudatus* are spatially heterogeneous at the skin, but become increasingly homogeneous with distance (Stoddard et al. 1999). Thus a free-swimming female *B. pinnicaudatus* might assess a male's EOD amplitude without contacting him directly.

EOD amplitude is the most likely cue used by females in our study to discriminate between prospective mates, although it is possible that females may use another cue,
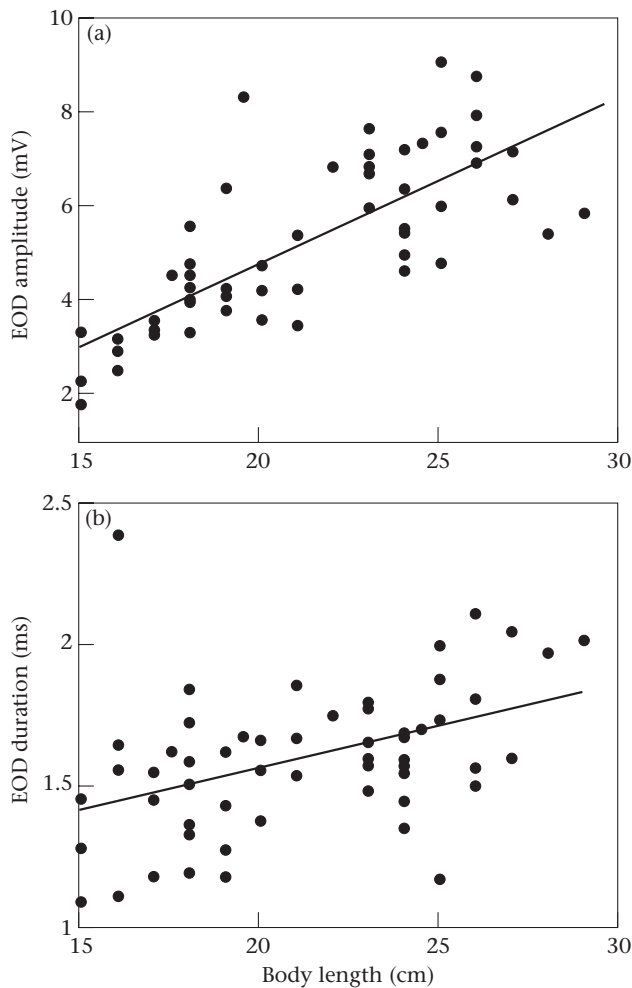
Figure 5. Regression of total body length against EOD parameters for male *B. pinnicaudatus* in test 3 (*N*=56). Body length was a good predictor of (a) EOD peak-to-peak amplitude (r^2=0.58) but only a fair predictor of (b) EOD duration (r^2=0.19).

perhaps one not measured in our study. For example, female swordtail fish, *Xiphophorus nigrensis*, prefer larger males not only because they are larger, but also because they engage in more courtship behaviour (Ryan et al. 1990). Likewise, in other taxa, such as grey tree frogs, *Hyla versicolour*, and sage grouse, *Centrocercus urophasianus*, females appear to be sensitive to the amount of energy invested by males in their courtship displays (Gibson & Bradbury 1985; Klump & Gerhardt 1987).

Males in our test groups were seventh- and eighth-generation captive-bred fish from a small founder population. We assumed, therefore, that they would show little heritable variation in phenotype. None the less, they varied considerably in size and EOD characters due to uncontrolled variation in age, competition and food availability in the rearing pools. By controlling the amount of food available in the rearing pools we have found that we can control male size at sexual maturity. Male phenotypic variation in the wild is probably also influenced by a variety of genetic factors related to foraging ability, pathogen resistance and growth rate. We do

not know whether growth rate or body size of males is polymorphic within a population of *B. pinnicaudatus*.

A female's preference for male phenotype under captive conditions does not exclude her reliance on other cues under field conditions. Female swordtail fish make active choices between males in captivity, but under natural conditions, competition among males contributes additional factors (Ryan et al. 1990; Morris et al. 1992). Captive female song sparrows, *Melospiza melodia*, show sexual behaviour in response to song playbacks, but females in the field appear to choose mates based on other factors, such as shrub cover defended by the male (Searcy 1984; Arcese 1990). In this study, we controlled the quality of oviposition sites so that the only differences were the males themselves. In the field, differences in oviposition site quality may affect choice. In our outdoor breeding pools, females oviposit only in healthy, bushy plants that provide the best protection to their eggs. Thus, male competition for high-quality oviposition sites (classic resource defence polygyny, Emlen & Oring 1977) could affect the ultimate outcome of female mate decisions in *B. pinnicaudatus*. Hagedorn & Zelick (1989) reported that relative dominance between male *B. occidentalis* is expressed in the EOD spectrum. Franchina et al. (2001) found that male *B. pinnicaudatus* increased their EOD amplitudes more quickly in the presence of males than females. Both of these findings are consistent with a role of intrasexual competition on the male EOD. For these reasons, female choices of male phenotype do not exclude the possibility that intramale competition is an important indirect force in mate selection in hypopomid electric fish.

Female *B. pinnicaudatus* consistently preferred large males in both a sequential test paradigm (test 1: male versus female) and in a best-of-two paradigm (tests 2, 3: large male versus small male). In test 1, the females may have had a minimum threshold criterion, rejecting smaller males even when no larger male was available. In fact, females were more likely to move to the female side than to the side with a small male. Kramer & Otto (1988) found that both sexes of the gymnotiform electric fish *Eigenmannia* showed a preference for a female EOD playback, suggesting that the females in that study may have been disinterested in spawning. The apparent choice of a female over a small male may not be unusual if the test female finds the stimulus male unacceptable and actively avoids him. Female *B. pinnicaudatus* display a suite of behaviours for rejecting unwanted suitors, the most common of which include fleeing the male's presence or silencing their own EODs while remaining nearby (P. K. Stoddard, M. Kilburn & K. Patterson, unpublished data). Such behaviours are consistent with a 'best of *N*' selection strategy where males are dispersed over a region too large to permit simultaneous comparison by a female. The high incidence of nonmovement on any given night suggests that females do not approach males randomly, but approach only particular males that are within an acceptable phenotype.

The encouraging results of this study beg the obvious question of whether female *B. pinnicaudatus* will choose between playbacks of male EODs. Two playback

experiments, in which the repetition rate was held constant but the duration and amplitude were manipulated, have not yielded consistent results (Curtis 1999). One yielded a nonsignificant result of 10 larger male signals chosen and eight smaller male signals chosen. The other yielded six larger male signals chosen and 12 smaller male signals chosen. Simple EOD playbacks lack both the dynamic temporal interactions and the spatial heterogeneity of natural EODs, either of which might be necessary to elicit a meaningful response by the female. Our next approach will include these clues.

Acknowledgments

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Appendix

Table A1. Body length and EOD measurements of males used in test 3

Female ID	Chosen male ID	Body length (cm)	EOD duration (ms)	EOD amplitude (mV)	Rejected male ID	Body length (cm)	EOD duration (ms)	EOD amplitude (mV)
1	74	24.0	1.58	5.50	75	20.0	1.56	4.79
2	33	16.0*	1.12*	2.55*	32	21.0	1.68	3.51
3	13	24.0	1.69	5.02*	12	19.5	1.68	8.37
8	36	23.0	1.78	6.03	37	16.0	1.65	2.96
9	38	24.0	1.46	4.68	39	19.0	1.19	4.14
10	27	22.0	1.76	6.90	26	17.0	1.19	3.30
11	47	19.0*	1.44	6.45	46	25.0	1.18	6.07
12	78	24.0	1.36*	6.45	79	16.0	1.56	3.22
13	24	24.0	1.55	6.44	25	20.0	1.38	4.25
15	42	21.0	1.86	5.44	43	15.0	1.29	3.34
16	23	28.0	1.99	5.50	22	21.0	1.55	4.31
18	21	23.0	1.60	6.87	20	17.0	1.55	3.38
19	19	18.0*	1.20*	3.36*	18	26.0	2.12	7.01
20	11	25.0	1.74	7.65	10	18.0	1.59	4.04
21	16	18.0*	1.85*	4.31*	17	25.0	2.01	9.14
22	14	17.5*	1.63*	4.59*	15	24.5	1.71	7.42
23	41	23.0	1.49	7.18	40	18.0	1.34	5.63
24	59	16.0*	2.39	2.96*	58	24.0	1.70	5.56
25	29	24.0	1.60*	7.28	28	18.0	1.73	4.01
26	61	27.0	1.61*	7.26	60	19.0	1.63	4.29
27	63	23.0	1.66	6.90	62	17.0	1.46	3.61
28	71	23.0	1.58	7.73	70	18.0	1.51	4.82
29	67	15.0*	1.46*	1.81*	66	26.0	1.58	7.35
30	68	27.0	2.06	6.22	69	15.0	1.09	2.31
31	80	29.0	2.03	5.94*	81	23.0	1.80	6.76
32	83	19.0*	1.28*	3.83*	82	26.0	1.51	8.84
33	84	25.0	1.89	4.86	85	18.0	1.37	4.59
34	86	26.0	1.82	8.02	87	20.0	1.67	3.64

Asterisks mark measurements in which the chosen male was inferior to the rejected male.