

Structure and sexual dimorphism of the electrocommunication signals of the weakly electric fish, *Adontosternarchus devenanzii*

Muchu Zhou¹ and G. Troy Smith^{1,2,3,*}

¹Department of Biology, ²Center for the Integrative Study of Animal Behavior and ³Program in Neuroscience, Indiana University, Bloomington, IN 47405, USA

*Author for correspondence (e-mail: getsmith@indiana.edu)

Accepted 3 October 2006

Summary

Electrocommunication signals of electric fish vary across species, sexes and individuals. The diversity of these signals and the relative simplicity of the neural circuits controlling them make them a model well-suited for studying the mechanisms, evolution and sexual differentiation of behavior. In most wave-type gymnotiform knifefishes, electric organ discharge (EOD) frequency and EOD modulations known as chirps are sexually dimorphic. In the most speciose gymnotiform family, the Aptereronotidae, EOD frequency is higher in males than females in some species, but lower in males than females in others. Sex differences in EOD frequency and chirping, however, have been examined in only three apteronotid species in a single genus, *Aptereronotus*. To understand the **diversity** of electrocommunication signals, we characterized these behaviors in another genus, *Adontosternarchus*. Electrocommunication signals of *Adontosternarchus devenanzii* differed from those of *Aptereronotus* in several ways. Unlike in *Aptereronotus*, EOD frequency was not sexually dimorphic in *A. devenanzii*.

Furthermore, although *A. devenanzii* chirped in response to playbacks simulating conspecific EODs, the number of chirps did not vary with different stimulus frequencies. *A. devenanzii* chirps also differed in structure from *Aptereronotus* chirps. Whereas *Aptereronotus* species produce functionally distinct chirp types differing in frequency modulation (FM), *A. devenanzii* produced only high-frequency chirps that had either single or multiple frequency peaks. Males produced more multi-peaked chirps than females. Thus, the temporal structure of chirps, rather than the amount of FM, delineated chirp types in *A. devenanzii*. Our results demonstrate that the structure, function and sexual dimorphism of electrocommunication signals are evolutionarily labile in apteronotids and may be useful for understanding the **diversity** of sexually dimorphic behavior.

Key words: communication, sexual dimorphism, electric fish, signal evolution, *Adontosternarchus devenanzii*.

Introduction

Reproductive and agonistic communication signals are among the most conspicuous and diverse of animal behaviors. These signals vary both across and within species, are often highly sexually dimorphic and can therefore serve as models for understanding the evolution of behavioral **diversity** and the mechanisms that regulate sex differences in behavior.

The electrocommunication signals of weakly electric fish provide an opportunity to study the mechanisms and evolution of diversity in sexually dimorphic communication. Both African mormyriiform and Neotropical gymnotiform fishes possess electric organs whose weak electrical discharges function in electrolocation and communication. The properties of electric organ discharges (EODs) differ between species and can also vary as a function of sex, reproductive condition and/or social rank (Bass, 1986; Carlson et al., 2000; Dunlap and Larkins-Ford, 2003; Franchina et al., 2001; Hagedorn and

Heiligenberg, 1985; Hopkins, 1988; Kramer et al., 1980; Zakon and Smith, 2002). Each species produces one of two types of discharge: pulse-type or wave-type EODs. In pulse-type EODs, the duration of each discharge is much shorter than the time between discharges, whereas the duration of each discharge for wave-type EODs is approximately the same as the time between discharges, resulting in a quasi-sinusoidal signal (reviewed by Hopkins, 1988; Moller, 1995).

In species that produce wave-type EODs, the frequency of the discharge (i.e. number of discharges per second) often differs between the sexes. In most of the wave-type gymnotiform fish that have been studied, males emit lower frequency EODs than females (Dunlap and Zakon, 1998; Hagedorn and Heiligenberg, 1985; Hopkins, 1974b). Interestingly, however, in the most speciose gymnotiform family, the Aptereronotidae, sex differences in EOD frequency have been studied in only three species in a single genus, and

the direction of sexual dimorphism differs between these species. In the black ghost knifefish (*Apteronotus albifrons*), males produce EODs at significantly lower frequencies than females, whereas in two closely related species commonly called brown ghost knifefish (*Apteronotus leptorhynchus* and *Apteronotus rostratus*), EOD frequency is higher in males than females (Dunlap et al., 1998; Hagedorn and Heiligenberg, 1985; Kolodziejski et al., 2005; Meyer et al., 1987). Although the hormonal mechanisms underlying this reversal in the direction of sexual dimorphism in EOD frequency have been studied (Dunlap et al., 1998), the function of males having higher *versus* lower EOD frequency than females in apteronotids is not known.

Another type of electrocommunication signal, chirping, also differs across species and between sexes. Wave-type EODs are continuously emitted at precise frequencies that can indicate species, sex and/or rank. When fish interact, however, they can also transiently modulate the frequency and/or amplitude of their EODs to produce different types of signals known as chirps, gradual frequency rises (GFRs) and interruptions (Dye, 1987; Hagedorn and Heiligenberg, 1985; Hopkins, 1974b; Larimer and MacDonald, 1968). In *A. leptorhynchus*, chirping is highly sexually dimorphic, with males chirping more than females (Dunlap et al., 1998; Kolodziejski et al., 2005; Zupanc and Maler, 1993). By contrast, the amount of chirping is not sexually dimorphic in *A. albifrons* (Dunlap and Larkins-Ford, 2003; Dunlap et al., 1998; Kolodziejski et al., 2005).

The structure of chirps [i.e. the duration and degree of amplitude and frequency modulation (FM)] also varies between sexes and across species. Although *A. leptorhynchus* and *A. albifrons* both produce similar types of chirps, the chirps of *A. albifrons* are approximately 10 times longer in duration than comparable chirp types in *A. leptorhynchus* (Dunlap and Larkins-Ford, 2003; Kolodziejski et al., 2005). High-frequency chirps (i.e. chirps with more than 150 Hz of FM) are produced more often by males than females in both species, and the amount of FM and/or duration of chirps is also sexually dimorphic (Dunlap and Larkins-Ford, 2003; Dunlap et al., 1998; Hagedorn and Heiligenberg, 1985; Kolodziejski et al., 2005).

Thus, the closely related apteronotid species whose electrocommunication signals have been well-studied differ in the degree and/or direction of sexual dimorphism in EOD frequency and chirping. Since more than 60 apteronotid species in 14 genera have been identified (Crampton and Albert, [in press](#)) and because electrocommunication signals can be easily recorded and quantified, this family offers an unusual opportunity to investigate the evolution of sexually dimorphic communication. To take advantage of this species diversity, however, the communication signals of apteronotid fish in genera other than *Apteronotus* must be studied. We further characterized the diversity of electrocommunication signals by examining the structure of chirps and sex differences in EOD frequency and chirping in *Adontosternarchus devenanzii*, an apteronotid species in a genus with numerous derived characters, including intraspecific diversity in EOD waveform

and the presence of accessory electric organs (Bennett, 1971; Crampton and Albert, 2006).

Materials and methods

Subjects, housing and assessment of sex and reproductive condition

Adontosternarchus devenanzii (♂) (11 males and 10 females) were purchased from a reputable commercial supplier (Rose Tropical Fish, Miami, FL, USA) and were housed in 65 l or 34 l tanks maintained at 26–27°C, pH 5.5–6.5 and conductivity of 100–500 $\mu\text{S cm}^{-1}$. Experiments complied with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and protocols approved by the Indiana University Animal Care and Use Committee. *A. devenanzii* is not sexually dimorphic in body size or external morphology, and we were therefore unaware of the sex of each fish when its electrocommunication behavior was recorded. The sex of most fish was determined later by laparotomy. After behavioral testing was completed, fish were anesthetized with 0.075% 2-phenoxyethanol. A small incision was made in the ventral body wall, and the gonads were examined to determine the sex of the fish. The incision was sutured with 8-0 silk and sealed with Nexaband surgical tissue adhesive (Abbott Laboratories, North Chicago, IL, USA). One male and one female died after the study, and two males and two females were killed after their behavioral recordings for use in a separate immunohistochemical study. The sex of these fish was determined by post-mortem examination of the gonads. In these cases, gonads were removed and weighed, and reproductive condition was estimated by calculating the gonadosomatic index (GSI, gonad mass \times 100/body mass). Some of the fish (five males and six females) were weighed to the nearest 0.1 g to test for sex differences in body mass and/or correlations between size and EOD frequency or chirping.

Recording electrocommunication behavior

The EOD frequency of each fish was measured by placing a shielded pair of wires next to the tail, amplifying the voltage between those wires (100 \times ; model P-55; Grass Instruments, W. Warwick, RI, USA) and using the frequency counter of a digital multimeter (Fluke model 187, Everett, WA, USA). The temperature of the water was also measured to the nearest 0.1°C, and a Q_{10} of 1.8 was used to correct each EOD frequency measurement to that expected at 26.0°C (Dunlap et al., 2000).

EOD modulations were recorded and analyzed by using methods described previously (Kolodziejski et al., 2005). Briefly, fish were placed in a PVC tube with plastic mesh over both ends and a mesh-covered window midway down the length of the tube. The tube was placed in the center of a 37 l aquarium maintained at 25.8–27.0°C and at the conductivity and pH of the fish's home tank. The fish were allowed to acclimate to the recording tank for 1 h. A pair of carbon electrodes placed at the fish's head and tail recorded its EOD,

and a second pair of electrodes on either side of the tube was used to present playback stimuli. The signal from the recording electrodes was band-pass filtered (0.1 Hz–10 kHz), amplified (100–1000 \times ; Grass model P-55) and digitized at 44.1 kHz on the left channel of a sound card in a computer running Cool Edit Pro (Syntrillium, Phoenix, AZ, USA). Playback stimuli were sinusoidal voltage signals generated by a function generator (Model GFG-8216A or GFG-8219A; Instek, Chino, CA, USA) and calibrated to a root-mean-square field amplitude of 1.5 mV cm⁻¹ parallel to the stimulating electrodes and midway between them. This amplitude approximates that of the EOD of a medium-sized *A. devenanzii*. A copy of the stimulus was digitized on the right channel of the sound card. A 4-min baseline recording was made from each fish without stimulation, and five recordings were made with different playback stimuli. Each recording consisted of a 1-min baseline period with no stimulation, two minutes of playback stimulation and 1 min post-stimulus. The frequencies of the playback stimuli were set relative to the fishes' own EOD frequencies: 150 Hz above and below the EOD frequency (± 150 Hz), 20 Hz above and below the EOD frequency (± 20 Hz) and 5 Hz below the EOD frequency (-5 Hz). The playback frequencies spanned the species-typical range of EOD frequencies and were meant to simulate the presence of a conspecific fish in the recording tank. Based on results in other apteronotid fish, we expected the -5 Hz stimulus to evoke a jamming avoidance response (JAR) (Bullock et al., 1972). Stimuli were presented in random order and were separated by 10-min intervals without stimulation.

Analysis of EOD modulations

We used a customized procedure written by Brian Nelson (University of Oregon, Eugene, OR, USA) and running in Igor Pro (Wavemetrics, Lake Oswego, OR, USA) to calculate EOD frequency and to count and measure the parameters of EOD modulations (for details, see Kolodziejcki et al., 2005). Briefly, any playback-induced contamination of the recording was removed by subtracting an appropriately scaled and phase-shifted copy of the playback signal. The fundamental frequency of the EOD was calculated by using an autocorrelation algorithm on 6 ms Hanning windows, advanced 2 ms per iteration. This process resulted in a temporal resolution of 2 ms and a frequency resolution of 0.5–3 Hz, depending on the signal-noise ratio of the recording. The Igor procedure used the mode of EOD frequency in sliding 2 s windows as a baseline frequency from which to detect EOD modulations. The procedure counted EOD modulations as any event in which EOD frequency exceeded this baseline frequency by more than 3 Hz for more than 10 ms and less than 60 s. The beginning and end of each EOD modulation was then defined as the time at which EOD frequency crossed a threshold of 1 Hz above or below the baseline frequency. The procedure then calculated the duration and peak frequency of each modulation. Each EOD modulation was also examined by the experimenter to confirm

that the procedure accurately identified the EOD modulation and measured its parameters.

Statistics

Body mass, EOD frequency and the numbers and parameters of different types of EOD modulations were compared between males and females by using unpaired *t*-tests. To avoid pseudoreplication, we calculated mean² parameter values (FM and duration) for different EOD modulation types for each fish, and performed statistical analyses on these values². Repeated-measures analysis of variance (RM-ANOVA), with sex as an independent variable and stimulus frequency as the repeated measure, was used to determine whether the production of different² types of EOD modulations was influenced by the frequency of the playback stimulus. Since all fish received the same set of stimuli and stimulus frequency did not affect the production of EOD modulations (see Results), we analyzed pooled data for all of the EOD modulations that each individual produced during all six 4-min recordings (five recordings with stimuli and one baseline recording). Pearson's correlations were used to test for correlations between body mass and EOD frequency and numbers of EOD modulations. ~~$P=0.05$ was used for all statistical tests.~~

Results

Reproductive condition and body mass

The fish in this study were sexually mature and had well-developed gonads. We were only able to measure the GSI in six fish that died or were killed for use in a separate pilot study. The GSI was 0.34 ± 0.14 (0.135–0.614, $N=3$) in the males and 1.47 ± 0.47 (0.725–2.35, $N=3$) in the females. These values are similar to those in previous studies that found sex differences in the electrocommunication signals of other apteronotid species (Dunlap et al., 1998; Kolodziejcki et al., 2005). Visual inspection of the gonads in the laparotomized fish also demonstrated that the gonads were well-developed (e.g. yolked follicles were present in females) and that the reproductive condition of these fish was comparable to that of the fish whose GSI values were measured. All of the fish were adults, and total body length for the fish used in this study (180.1–219.0 mm) was at the top end of the range of lengths recorded in the holotype and paratypes of *A. devenanzii* (Mago-Leccia et al., 1985). The size of males and females overlapped considerably, and there was no sex difference in body mass (Table 1, $t_9=0.40$, $P=0.70$).

EOD frequency

EOD frequencies ranged from 917 to 1168 Hz at 26.0°C. In contrast to *A. leptorhynchus* and *A. albifrons*, in which EOD frequency is highly sexually dimorphic (Dunlap and Larkins-Ford, 2003; Kolodziejcki et al., 2005; Meyer, 1983), EOD frequency in *A. devenanzii* did not differ significantly between males and females ($t_{19}=1.78$, $P=0.091$), although males tended to have slightly lower EOD frequencies than females (Table 1). EOD frequency was not significantly correlated with body mass ($r^2=0.11$, $P=0.33$)

Table 1. Summary of sex differences in physical traits, EOD frequency and EOD modulations

Trait	Males	Females ¹
Body mass (g)	34.8±5.7	32.2±3.3
EOD frequency (Hz) ²	1052.6±22.7	1101.4±14.4
EOD modulations (EODMs, chirps + GFRs) ³ :		
Total number (EODMs 24 min^{-1}) ⁴	4.6±4.5	14.0±3.3
By type ³ :		
All chirps (single- and multi-peaked)		
Number produced (chirps 24 min^{-1})	18.3±4.2	6.8±2.8 ⁵
% of total EODMs	68.3±7.9	40.0±9.5*
Positive FM (Hz)	170.7±10.6	167.3±12.2
Duration (s)	0.090±0.011	0.060±0.021
Multi-peaked chirps (chirps 24 min^{-1})	6.0±2.1	0.4±0.3*
All gradual frequency rises (GFRs)		
Number produced (GFRs 24 min^{-1})	6.3±1.2	6.6±1.5
% of total EODMs	31.7±7.9	60.0±9.5*
Positive FM (Hz)	12.8±2.4	8.2±1.2
Duration (s)	1.06±0.33	0.75±0.48
Multi-peaked GFRs (GFRs 24 min^{-1})	2.3±0.5	1.6±0.5

Values are means ± s.e.m.

*Statistically significant sex difference; unpaired *t*-test, $P < 0.05$.

¹For body mass, $N=5$ males, 6 females. For EOD frequency, $N=11$ males, 10 females. For EOD modulations, $N=11$ males, 8 females.

²Temperature compensated to that expected at 26.0°C (see Materials and methods).

³See Fig. 1, Materials and methods and Results for a description of EOD modulation types.

⁴Since stimulus frequency did not affect the production of EODMs (see Fig. 4 and Results) and all fish received the same set of stimuli, data are pooled from all six 4-min recordings (five recordings with different stimuli and one baseline recording).

⁵Unpaired *t*-test (males versus females), $P=0.053$.

Structure and types of EOD modulations

As in other apteronotids, *A. devenanzii* produced both chirps and GFRs (Fig. 1). The FM (increase in EOD frequency) of chirps ranged from 90 to 404 Hz, with most chirps having 100–250 Hz of FM. Chirp durations ranged from 18 ms to 2 s, although most chirps were 20–150 ms long. GFRs typically had much less FM (3–100 Hz, interquartile range 4.6–11.1 Hz) and longer and more variable duration (14 ms–15 s, interquartile range 32–264 ms). In *A. leptorhynchus* and *A. albifrons*, chirps can be unambiguously placed into two broad categories: high-frequency chirps with greater than 150 Hz of FM and low-frequency chirps with ~30–100 Hz of FM (Fig. 1B) (Bastian et al., 2001; Engler et al., 2000; Hagedorn and Heiligenberg, 1985; Kolodziejwski et al., 2005). The chirps of *A. devenanzii* could not be placed into clear categories based on the amount of FM. The FM of *A. devenanzii* chirps was most similar to that of the high-frequency chirps of *A. leptorhynchus* and *A. albifrons* (i.e. typically greater than 100 Hz of FM), and no

low-frequency chirps were produced. Both the chirps and GFRs of *A. devenanzii*, however, did vary systematically in another parameter: the number of frequency peaks. Although some chirps and GFRs had a single frequency peak, similar to that in most chirps produced by *A. leptorhynchus* and *A. albifrons*, many *A. devenanzii* chirps (26.6%) and GFRs (33%) had multiple frequency peaks (Fig. 1A,E). Most multi-peaked chirps had 2–4 peaks, although a few had as many as nine peaks. Interestingly, although multi-peaked chirps typically had greater mean² duration than single-peaked chirps, the duration of single-peaked chirps was more variable than that of multi-peaked chirps, and the longest chirps were single-peaked rather than multi-peaked (Fig. 1A,C,D). Unlike the high-frequency chirps of *A. leptorhynchus*, but similar to those in *A. albifrons*, the chirps of *A. devenanzii* lacked frequency undershoots (i.e. a decrease in EOD frequency below its baseline) at the end of the chirp. Similarly, chirps in both *A. devenanzii* and *A. albifrons* had durations several times longer than those of *A. leptorhynchus* (Fig. 1; Table 1) (Dunlap and Larkins-Ford, 2003; Kolodziejwski et al., 2005).

Sex differences in EOD modulations

EOD modulations were sexually dimorphic in *A. devenanzii*, but these sex differences were less pronounced than those in *A. leptorhynchus* (Table 1; Fig. 2). Although males tended to produce more chirps than females, this difference did not reach statistical significance ($t_{17}=2.08$, $P=0.053$). The proportion of EOD modulations that were chirps (as opposed to GFRs), however, was significantly greater in males than females; on average, 68.3% of the male EOD modulations were chirps, compared with only 40% of the female EOD modulations ($t_{17}=2.30$, $P=0.035$). Most of the multi-peaked chirps were produced by males; males produced more than 12 times as many multi-peaked chirps as females ($t_{17}=2.30$, $P=0.035$). There were no sex differences in the number of GFRs, and neither the FM nor the duration of chirps or GFRs differed significantly between males and females (unpaired *t*-tests, $P > 0.15$ for all).

The production of EOD modulations was not related to individual variation in size. Body mass was not significantly correlated with the number of total EOD modulations ($r^2 < 0.01$, $P=0.94$), chirps ($r^2 < 0.01$, $P=0.84$), GFRs ($r^2=0.07$, $P=0.42$), multi-peaked chirps ($r^2=0.05$, $P=0.51$) or multi-peaked GFRs ($r^2 < 0.01$, $P=0.78$).

Effect of stimulus frequency on EOD modulations

A. devenanzii did not chirp differently in response to different stimulus frequencies (Fig. 3). Neither the total number of chirps nor the number of multi-peaked chirps was affected by stimulus frequency (RM-ANOVA, main effect of stimulus frequency, $F_{4,68}=1.27$ and 1.02, $P > 0.29$). Consistent with the unpaired *t*-test demonstrating that males produced more multi-peaked chirps, the number of multi-peaked chirps was significantly affected by sex (RM-ANOVA, $F_{1,17}=5.15$, $P=0.037$); but the interactions of stimulus frequency and sex on the total number of chirps or multi-peaked chirps were not

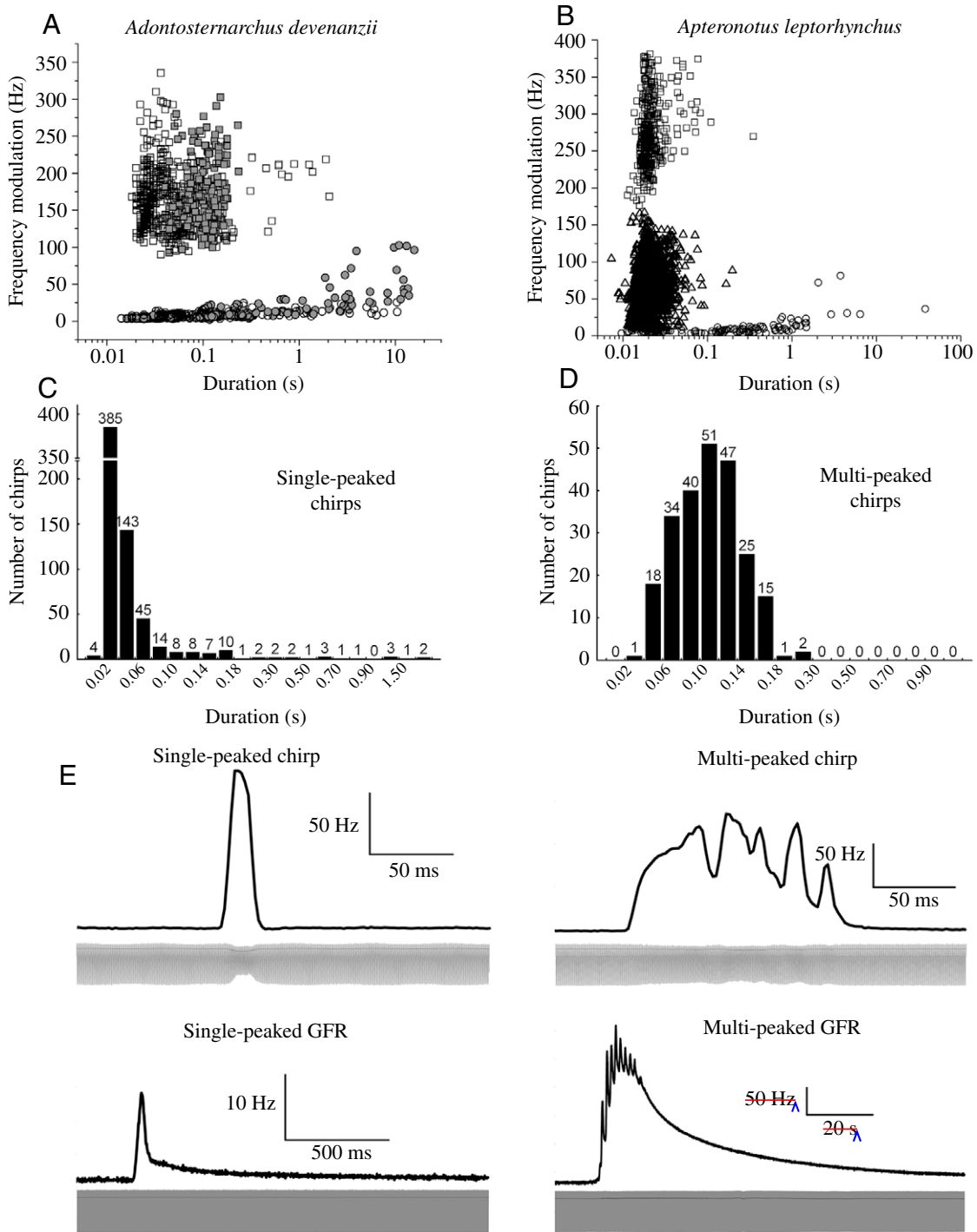


Fig. 1. EOD modulations in *A. devenanzii* (A,C–E) and *A. leptorhynchus* (B). (A) Scatter plot of the frequency modulation (FM, Hz) and duration (s) of 1286 EOD modulations recorded from 11 male and eight female *A. devenanzii*. Two types of EOD modulations could be distinguished based on the degree of FM: chirps (squares) and gradual frequency rises (GFRs, circles). Chirps and GFRs could have either single frequency peaks (open symbols) or multiple frequency peaks (grey symbols). Note that because so many chirps are plotted, many single-peaked chirps and GFRs are obscured by overlying multi-peaked chirps and GFRs. (B) A comparable plot for 7950 EOD modulations from *A. leptorhynchus* based on data from Kolodziejski et al. (Kolodziejski et al., 2005). *A. leptorhynchus* produces GFRs (circles) and two types of chirps: high-frequency chirps (squares) and low-frequency chirps (triangles). Note the absence of low-frequency chirps in *Adontosternarchus* and the lack of multi-peaked modulations in *A. leptorhynchus*. (C) Histogram of the duration of single-peaked chirps. Although most single-peaked chirps lasted 0.02–0.08 s, there is a ‘tail’ of longer duration chirps. (D) Histogram of the duration of multi-peaked chirps. (E) Examples of single- and multi-peaked chirps and GFRs produced by *A. devenanzii*. The top trace in each example tracks EOD frequency, and the bottom trace is the raw voltage record. Note the different time and frequency scales for chirps and GFRs.

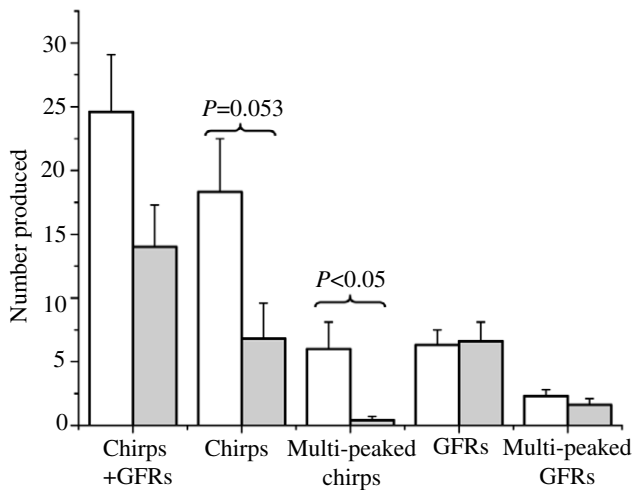


Fig. 2. Numbers of each type of EOD modulation produced (mean \pm s.e.m.) by male (white bars) and female (grey bars) *A. devenanzii*. The *P*-values for significant or marginally insignificant sex differences (unpaired *t*-tests) are indicated above the bars.

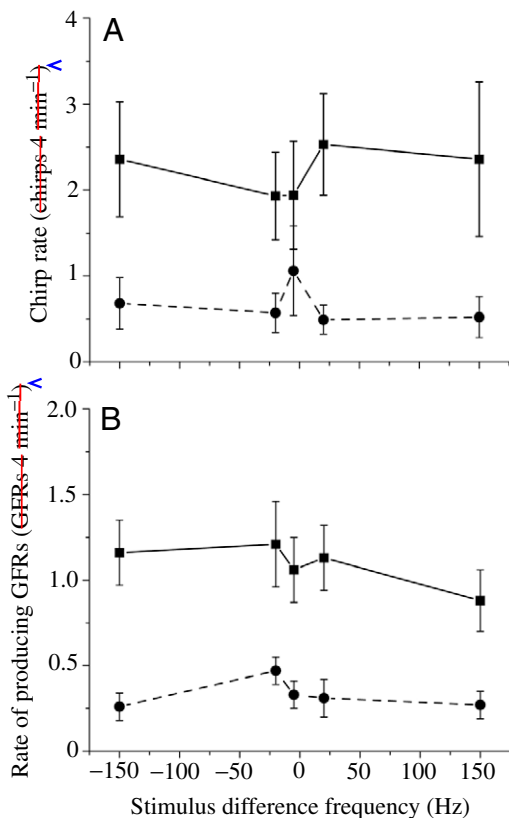


Fig. 3. Effect of stimulus difference frequency (i.e. the difference between the subject fish's EOD frequency and the stimulus frequency) on the number of chirps (A) and GFRs (B). The numbers of multi-peaked chirps and multi-peaked GFRs are indicated by broken lines, and the total numbers of chirps and GFRs by solid lines. Stimulus frequency did not significantly affect the number of any of these EOD modulations.

significant (RM-ANOVA, $F_{4,68}=0.74$ and 0.88 , $P>0.48$). Neither sex nor stimulus frequency affected the number of GFRs or multi-peaked GFRs (RM-ANOVA, $P>0.26$ for all factors).

As in other apteronotid species (Bullock et al., 1972), the -5 Hz difference frequency stimulus evoked a JAR. The magnitude of the JAR (i.e. the sustained increase in EOD frequency caused by the -5 Hz difference frequency stimulus) was not sexually dimorphic (males 5.48 ± 0.76 Hz, females 5.09 ± 1.11 Hz; $t_{16}=0.30$, $P=0.77$).

Discussion

The electrocommunication signals of *A. devenanzii* differed from those in other apteronotid species. EOD frequency was not sexually dimorphic, and sex differences in chirping were less pronounced than in *A. leptorhynchus*. Chirps could not be separated into types based on the amount of FM, but both chirps and GFRs could have either single or multiple frequency peaks. Multi-peaked chirps were predominantly produced by males. Finally, unlike other apteronotids, *A. devenanzii* did not chirp differently in response to playbacks of different frequencies.

Species diversity in the structure of EOD modulations

The two other apteronotid species whose electrocommunication behavior has been extensively studied produce three comparable types of EOD modulations (Kolodziejski et al., 2005). Both *A. leptorhynchus* and *A. albifrons* produce high-frequency chirps, with more than 150 Hz of FM; low-frequency chirps, with approximately 20–150 Hz of FM; and GFRs, which have much less FM and more variable duration. The main difference between the EOD modulations of these two *Apteronotus* species is that the chirps of *A. albifrons* last 8–12 times longer than those of *A. leptorhynchus* (Dunlap and Larkins-Ford, 2003; Kolodziejski et al., 2005). *A. devenanzii* also produced chirps and GFRs, but the chirps of *A. devenanzii* could not be categorized as high- and low-frequency chirps. The FM of *A. devenanzii* chirps (90–404 Hz) was most similar to the FM of high-frequency chirps in *Apteronotus*. Unlike *Apteronotus*, *A. devenanzii* never produced chirps with less than 90 Hz of FM (Fig. 1A). *A. devenanzii* also differed from the other apteronotids in the production of multi-peaked EOD modulations. Many of the chirps and GFRs in *A. devenanzii* had multiple frequency peaks. Although *A. albifrons* can produce multi-peaked high-frequency chirps, and *A. leptorhynchus* can produce extremely long-duration high-frequency chirps, these modulations are rare (Dunlap and Larkins-Ford, 2003; Engler and Zupanc, 2001). Similarly, although GFRs in all apteronotids are highly variable in duration and can have complex FM over time in *A. albifrons* (Serrano-Fernandez, 2003), GFRs with multiple sharp frequency peaks like those in *A. devenanzii* (Fig. 1E) have not been reported in other apteronotids. The species differences in chirp parameters suggest that the structure of chirps and GFRs and the differentiation of chirps into

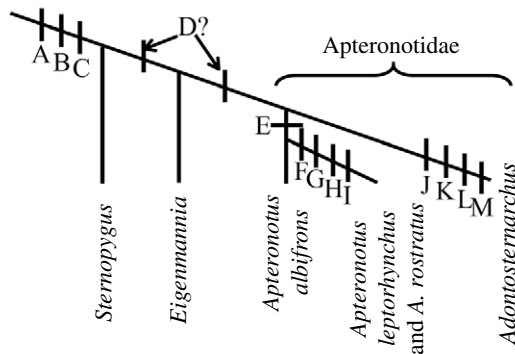


Fig. 4. Partial phylogeny of wave-type gymnotiform fish, illustrating evolution of electrocommunication signals and their sexual dimorphism. Phylogeny based on Crampton and Albert (Crampton and Albert, 2006). Comparison of electrocommunication signals based on this study and published reports (Hopkins, 1974b; Hopkins, 1974c; Meyer, 1983; Hagedorn and Heiligenberg, 1985; Dye, 1987; Zupanc and Maler, 1993; Dunlap and Zakon, 1998; Dunlap et al., 1998; Dunlap and Larkins-Ford, 2003; Kolodziejewski et al., 2005). ‘X’, presence of the trait; ‘O’, absence of the trait; ‘?’, either the trait has not been investigated or the data are equivocal. ¹Long- and short-duration interruptions in *Eigenmannia* may be analogous to high- and low-frequency chirps in *Apterotonotus* (Hagedorn and Heiligenberg, 1985; Hopkins, 1974c). ²*A. albifrons* can produce GFRs and chirps that have complex spectro-temporal structure (Dunlap and Larkins-Ford, 2003; Serrano-Fernandez, 2003), but they are rare and are not similar to the multi-peaked chirps of *A. devenanzii*. ³Hopkins recorded EOD modulations in only one female *Sternopygus* and it is thus unclear whether EOD modulations are sexually dimorphic in this genus (Hopkins, 1974b). ⁴Data from Hopkins (Hopkins, 1974c) and Hagedorn and Heiligenberg (Hagedorn and Heiligenberg, 1985) suggest sex differences in the number and/or structure of interruptions and rises, but statistical analyses were not reported. A, lower EOD frequencies in males than females; B, gradual frequency rises (GFRs); C, EOD modulations differentially depend on EOD frequencies of other fish; D, chirping; E, distinct high- and low-frequency chirps; F, higher EOD frequencies in males than females; G, sex difference in number of EOD modulations; H, short-duration chirps; I, high-frequency chirps with frequency undershoots; J, loss of sex difference in EOD frequency; K, multi-peaked chirps and GFRs common; L, more multi-peaked chirps produced by males; M, loss of differential production of EOD modulations based on EOD frequencies of other fish.

Sex difference in EOD frequency	♀>♂	♀>♂	♀>♂	♂>♀	♀=♂
GFRs	X	X	X	X	X
Interruptions	X	X	O	O	O
High-frequency chirps	O	? ¹	X	X	X
Low-frequency chirps	O	? ¹	X	X	O
Multi-peaked chirps and GFRs	O	O	O ²	O	X
Sex difference in chirp/GFR rate	? ³	? ⁴	♀=♂	♂>♀	♀=♂
Sex difference in chirp/GFR structure	? ³	? ⁴	X	X	X
Playback frequency affects chirping/GFRs	X	X	X	X	O

categories have changed during apteronotid evolution (Fig. 4). In *Apterotonotus*, distinct chirp types differ mainly in the degree of FM, whereas in *Adontosternarchus* chirp categories may be based on whether they are single- or multi-peaked. Previous studies have hypothesized distinct functions for different categories of chirps in *A. leptorhynchus*, with high-frequency chirps serving as courtship signals, low-frequency chirps as aggressive signals and GFRs as either submissive signals or ‘victory cries’ (Bastian et al., 2001; Dye and Heiligenberg, 1987; Engler and Zupanc, 2001; Hagedorn and Heiligenberg, 1985; Serrano-Fernandez, 2003). Additional comparative studies are needed to determine whether the function, as well as the structure, of different chirp types varies across apteronotid species.

Two chirp parameters in *A. devenanzii* were similar to those in *A. albifrons*, but differed from those in *A. leptorhynchus*. As in *A. albifrons*, *A. devenanzii* chirps lasted several times longer than most chirps in *A. leptorhynchus*. In addition, the high-frequency chirps of both *A. devenanzii* and *A. albifrons* lacked frequency undershoots. These results suggest that short-duration chirps and frequency undershoots are derived characters in *A. leptorhynchus* (Fig. 4). These two parameters could be mechanistically linked. Chirping is caused by glutamatergic excitation from the prepacemaker nucleus accelerating the firing rates of neurons in the pacemaker nucleus, the central pattern generator for the EOD. It is possible

that the rapid removal of excitation needed to produce short-duration chirps in *A. leptorhynchus* results in rebound hyperpolarization in the pacemaker neurons, reducing their firing rate and leading to a frequency undershoot. Such rebound hyperpolarization might not occur if the removal of excitation is more gradual, as would be expected for the longer duration chirps of *A. albifrons* and *A. devenanzii*. Consistent with this hypothesis, *A. leptorhynchus* rarely produces extremely long duration high-frequency chirps, which also lack frequency undershoots [Type 4 chirps of Engler and Zupanc (Engler and Zupanc, 2001)].

Further studies are needed to investigate the neural mechanisms underlying species differences in the structure of EOD modulations. In particular, what aspects of electromotor physiology allow the production of multi-peaked chirps and GFRs in *Adontosternarchus* but not *Apterotonotus*? One possibility is that projection neurons in the prepacemaker nucleus in *Adontosternarchus* fire in bursts and excite the pacemaker nucleus in an oscillatory pattern during EOD modulations, whereas those in *Apterotonotus* fire tonically. Alternatively, differences in chirp structure may result from species differences in postsynaptic responsiveness or intrinsic excitability of neurons in the pacemaker nucleus. For example, tonic glutamatergic excitation of pacemaker neurons by prepacemaker afferents might smoothly increase EOD frequency during chirps and GFRs in *Apterotonotus* but cause

oscillating FM in *Adontosternarchus*. Dunlap and Larkins-Ford similarly hypothesized that differences between *A. leptorhynchus* and *A. albifrons* in chirp duration might be mediated by postsynaptic mechanisms in the pacemaker nucleus (Dunlap and Larkins-Ford, 2003). The ability to study neuronal excitability by using *in vitro* preparations of the pacemaker nucleus (Dye, 1988; Smith and Zakon, 2000) and prepacemaker nucleus (G.T.S. and J.A. Kolodziejcki, unpublished observations) will allow these hypotheses to be tested.

EOD waveform and frequency vary considerably across species and may be used to identify conspecifics (Hopkins, 1974a; Kramer et al., 1980). The structure of EOD modulations, however, has been examined in relatively few species (Dunlap and Larkins-Ford, 2003; Hagedorn and Heiligenberg, 1985; Hopkins, 1974b; Hopkins, 1974c; Kolodziejcki et al., 2005). Our results and those of other studies suggest that the structure of EOD modulations may vary as much across species as EOD frequency, and thus may also convey species-identifying information.

Sex differences in EOD modulations

Sexual dimorphism of chirping varies across apteronotid species. Chirping is highly sexually dimorphic in *A. leptorhynchus*. Males chirp 20 to 40 times more than females, and high-frequency chirps are produced almost exclusively by males (Dunlap et al., 1998; Kolodziejcki et al., 2005; Zupanc and Maler, 1993). The number of chirps in *A. albifrons* is not sexually dimorphic, but chirp structure does differ between the sexes (Dunlap and Larkins-Ford, 2003; Dunlap et al., 1998; Kolodziejcki et al., 2005). Male *A. albifrons* produce more high-frequency chirps than females, and male chirps last longer than those of females. As in *A. albifrons*, the total number of EOD modulations was not sexually dimorphic in *A. devenanzii*, but males and females did differ in the types of chirps produced. Unlike the *Apteronotus* species, *A. devenanzii* did not produce distinct high- and low-frequency chirps, and chirps similar to the high-frequency chirps of *Apteronotus* were produced by both sexes. Male *A. devenanzii*, however, produced more than 10 times as many multi-peaked chirps as females. Thus, multi-peaked chirps, the electrocommunication signals that are most unique to *Adontosternarchus*, are also the most sexually dimorphic signals in *A. devenanzii*. This raises the interesting possibility that different chirp parameters have been sexually selected in different apteronotid lineages. In *Apteronotus*, high-frequency chirps are largely male-specific signals, whereas in *Adontosternarchus*, multi-peaked chirps are predominantly produced by males. Future studies examining the behavioral responses of fish to different types of chirps could test the hypothesis that the different types of electrocommunication signals produced mostly by males (i.e. high-frequency chirps in *Apteronotus* and multi-peaked chirps in *Adontosternarchus*) have evolved similar functions (e.g. courtship). Sexual selection for different signal parameters in closely related lineages has also been reported for other reproductive communication signals. For example, different

components of song have diversified through sexual selection in different congeneric songbird species and in different populations of a ring species (Irwin, 2000; Price and Lanyon, 2004).

Sex differences in EOD frequency

We found no significant sex difference in EOD frequency in *A. devenanzii*, even though, based on the GSI and the presence of yolked follicles in females, the fish in this study were sexually mature. EOD frequencies of males and females overlapped considerably. By contrast, EOD frequency in other apteronotid species differs markedly between males and females, with little or no overlap between the sexes (Dunlap et al., 1998; Hagedorn and Heiligenberg, 1985; Kolodziejcki et al., 2005; Meyer, 1983). Thus, the four species of apteronotids in which sex differences in EOD frequency have been examined display three distinct patterns of sexual dimorphism: (1) males have higher EOD frequencies than females in *A. leptorhynchus* and *A. rostratus*; (2) males have lower EOD frequencies than females in *A. albifrons*; and (3) EOD frequency is not sexually dimorphic in *A. devenanzii*. The diversity in the pattern of sex differences in the EOD in the few apteronotid species studied demonstrates that the direction and magnitude of sexual dimorphism in EOD frequency is evolutionary labile in this family.

In both *Sternopygus* and *Eigenmannia*, non-apteronotid gymnotiforms that also produce wave-type EODs, EOD frequency is lower in males than females (Dunlap and Zakon, 1998; Hagedorn and Heiligenberg, 1985) (Fig. 4) (Hopkins, 1974b; Zakon et al., 1991). It has thus been hypothesized that ancestral apteronotids also had males with lower EOD frequencies than females and that the reversal in the direction of sexual dimorphism of EOD frequency in *A. leptorhynchus* and *A. rostratus* is derived (Dunlap et al., 1998). Our results suggest that there may also have been a derived loss of sexual dimorphism in EOD frequency in the *Adontosternarchus* lineage (Fig. 4).

The interspecific variation not only in the presence or absence of sexual dimorphism of EOD frequency, but also in the direction of sex differences is unusual. Species differences in the magnitude of sexual dimorphism are common and may reflect differences in the relative strength of sexual and natural selection (Andersson, 1994). Although the direction of sexual dimorphism in body size also often varies (Fairbairn, 1997), species differences in the direction of sexual dimorphism in communication behavior, particularly in the absence of sex-role reversal, are rare. One example occurs in the parrot, *Electus roratus*, in which greater predation vulnerability in males and nest-site competition in females have favored females that are more brightly colored than males despite predominantly female parental care (Heinsohn et al., 2005).

Why does both the direction and degree of sexual dimorphism in EOD frequency vary across apteronotid species? In electric fish that produce pulse-type EODs, the waveform of the EOD is often sexually dimorphic.

Furthermore, sexual dimorphism of EOD waveform is typically in the same direction: males have longer duration, higher amplitude and/or more asymmetric EOD pulses than females (Hopkins, 1999). Sex differences in EOD waveform may be driven by strong directional sexual selection because the long duration, high amplitude and asymmetric EOD pulses of males require more energy to produce and/or make males more conspicuous to both females and electroreceptive predators (Hopkins, 1999; Stoddard, 1999; Stoddard, 2006). The relative costs and benefits of low- versus high-frequency EODs in wave-type electric fish are less clear. In fish that produce low-frequency EODs, each discharge lasts longer and thus may require more energy to produce (Mills and Zakon, 1987). However, because fish with low-frequency EODs produce fewer discharges per second, low-frequency EODs do not necessarily require more overall energy than high-frequency EODs (Hopkins, 1999). Furthermore, because capacitive coupling in the neurogenic electric organ of apteronotids strongly attenuates the **direct current (DC)** components of the EOD that are detectable by ampullary electroreceptors (Bennett, 1971), low-frequency EODs are unlikely to be any more or less conspicuous to electroreceptive predators than high-frequency EODs. If the costs and benefits of the EOD are not simply related to EOD frequency, the constraints underlying the directional sexual selection on the EOD in pulse-type electric fish may be relaxed in apteronotids, allowing diversification in the direction as well as the magnitude of sex differences in EOD frequency. Additional studies characterizing sex differences in EOD frequency in other gymnotiform species and determining whether the direction or magnitude of these sex differences is correlated with ecological factors (e.g. mating system, sociality, foraging ecology or predation) are needed to better understand the factors driving the evolution of sexually dimorphic EOD frequencies.

The physiological mechanisms underlying species diversity in electrocommunication behavior also require further investigation. The hormonal control of sex differences in EOD frequency has been characterized in *Apteronotus*. Consistent with the reversal in the direction of sexual dimorphism, androgens increase EOD frequency in *A. leptorhynchus*, but decrease EOD frequency in *A. albifrons* (Dunlap et al., 1998). The effects of hormones on electrocommunication signals in *Adontosternarchus* have not yet been studied, but one possible mechanism that could contribute to the lack of a sex difference in EOD frequency in this species would be an insensitivity of EOD frequency to gonadal steroids.

Sexual dimorphism in EOD frequency and differential responsiveness to playbacks

Sex differences in EOD frequency may be associated with responsiveness to playbacks of different frequencies. *A. leptorhynchus* produces more low-frequency chirps, which may function as agonistic signals, in response to playbacks of frequencies 5–10 Hz away from the fishes' own EOD than to more distant frequencies (Bastian et al., 2001; Engler and

Zupanc, 2001). Because EOD frequency is sexually dimorphic in *A. leptorhynchus*, more low-frequency chirps are thus produced in response to the EODs of same-sex than opposite-sex individuals. Furthermore, *A. leptorhynchus* males produce more high-frequency chirps, which may function in courtship, to playbacks with frequencies 50–200 Hz away from that of the male's own EOD (Bastian et al., 2001; Engler and Zupanc, 2001). Because female EOD frequencies are typically 100–200 Hz lower than those of males, this behavior results in males producing high-frequency chirps mostly in response to female EODs. *A. albifrons* also chirps differently in response to playbacks of different frequencies (J. A. Kolodziejski and G.T.S., unpublished observations). By contrast, we found no effect of stimulus frequency on the number or structure of chirps in *A. devenanzii*. The lack of an effect of stimulus frequency on chirping could be explained by the fact that EOD frequency in *A. devenanzii* is not sexually dimorphic and therefore does not convey information about sex. In both *A. leptorhynchus* and *A. albifrons*, sex differences in EOD frequency make it a reliable cue for directing chirps towards receivers of one sex or the other. By contrast, because EOD frequency does not differ between males and females in *A. devenanzii*, chirping differently to EODs of different frequencies would not necessarily direct chirps at individuals based on their sex. If *A. devenanzii* direct their chirps in a sex-specific manner, they may use cues other than EOD frequency to assess the sex of potential receivers.

Apteronotid electrocommunication as a model for studying the evolution of sexually dimorphic behavior

The results of this study and previous studies in other apteronotid species demonstrate abundant diversity in electrocommunication behavior (Fig. 4). Phylogenetic comparative methods to more thoroughly investigate the evolution of this diversity will require the characterization of sex differences in the electrocommunication behavior in additional species. The relative ease with which electrocommunication signals can be elicited and analyzed will facilitate this process. Furthermore, because electric fish respond robustly to playbacks with both electrical (e.g. chirping) and physical behaviors (e.g. by attacking electrodes or depositing eggs near electrodes playing back conspecific EODs), this system can be used to study the evolution of signal perception as well as production (Dye, 1987; Hagedorn and Heiligenberg, 1985; Hopkins, 1974c). Finally, the simplicity of neural circuits that control both the EOD and its modulations (Heiligenberg et al., 1996; Smith, 1999; Zakon and Smith, 2002) will allow comparative studies to investigate how sexually dimorphic behaviors and the physiological mechanisms that control them evolve together.

The authors thank Johanna Kolodziejski for technical assistance and Laura Hurley and an anonymous reviewer for comments on an earlier version of the manuscript. Supported by NIH MH 066960.

References

- Andersson, M. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.
- Bass, A. H. (1986). A hormone-sensitive communication system in an electric fish. *J. Neurobiol.* **17**, 131-156.
- Bastian, J., Schniederjan, S. and Nguyenkim, J. (2001). Arginine vasotocin modulates a sexually dimorphic communication behavior in the weakly electric fish *Apteronotus leptorhynchus*. *J. Exp. Biol.* **204**, 1909-1923.
- Bennett, M. V. L. (1971). Electric organs. In *Fish Physiology*. Vol. 5 (ed. W. S. Hoar and D. J. Randall), pp. 347-391. New York: Academic Press.
- Bullock, T. H., Hamstra, R. H. and Scheich, H. (1972). The jamming avoidance response of high frequency electric fish. I. General features. *J. Comp. Physiol.* **77**, 1-22.
- Carlson, B. A., Hopkins, C. D. and Thomas, P. (2000). Androgen correlates of socially induced changes in the electric organ discharge waveform of a mormyrid fish. *Horm. Behav.* **38**, 177-186.
- Crampton, W. G. R. and Albert, J. S. (2006). Evolution of electric signal diversity in gymnotiform fishes. In *Communication in Fishes*. Vol. 3 (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 23-40. Enfield, NH: Science Publishers.
- Dunlap, K. D. and Larkins-Ford, J. (2003). Diversity in the structure of electrocommunication signals within the genus of electric fish, *Apteronotus*. *J. Comp. Physiol. A* **189**, 153-161.
- Dunlap, K. D. and Zakon, H. H. (1998). Behavioral actions of androgens and androgen receptor expression in the electrocommunication system of an electric fish, *Eigenmannia virescens*. *Horm. Behav.* **34**, 30-38.
- Dunlap, K. D., Thomas, P. and Zakon, H. H. (1998). Diversity of sexual dimorphism in electrocommunication signals and its androgen regulation in a genus of electric fish, *Apteronotus*. *J. Comp. Physiol. A* **183**, 77-86.
- Dunlap, K. D., Smith, G. T. and Yekta, A. (2000). Temperature dependence of electrocommunication signals and their underlying neural rhythms in the weakly electric fish, *Apteronotus leptorhynchus*. *Brain Behav. Evol.* **55**, 152-162.
- Dye, J. (1987). Dynamics and stimulus-dependence of pacemaker control during behavioral modulations in the weakly electric fish, *Apteronotus*. *J. Comp. Physiol. A* **161**, 175-185.
- Dye, J. (1988). An in vitro physiological preparation of a vertebrate communicatory behavior: chirping in the weakly electric fish *Apteronotus*. *J. Comp. Physiol. A* **163**, 445-458.
- Dye, J. and Heiligenberg, W. (1987). Intracellular recording in the medullary pacemaker nucleus of the weakly electric fish, *Apteronotus*, during modulatory behaviors. *J. Comp. Physiol. A* **161**, 187-200.
- Engler, G., Fogarty, C. M., Banks, J. R. and Zupanc, G. K. (2000). Spontaneous modulations of the electric organ discharge in the weakly electric fish, *Apteronotus leptorhynchus*: a biophysical and behavioral analysis. *J. Comp. Physiol. A* **186**, 645-660.
- Engler, G. and Zupanc, G. K. (2001). Differential production of chirping behavior evoked by electrical stimulation of the weakly electric fish, *Apteronotus leptorhynchus*. *J. Comp. Physiol. A* **187**, 747-756.
- Fairbairn, D. J. (1997). Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annu. Rev. Ecol. Syst.* **28**, 659-687.
- Franchina, C. R., Salazar, V. L., Volmar, C.-H. and Stoddard, P. K. (2001). Plasticity of the electric organ discharge waveform of male *Brachyhypopomus pinnicaudatus*. II. Social effects. *J. Comp. Physiol. A* **187**, 45-52.
- Hagedorn, M. and Heiligenberg, W. (1985). Court and spark: electric signals in the courtship and mating of gymnotoid fish. *Anim. Behav.* **33**, 254-265.
- Heiligenberg, W., Metzner, W., Wong, C. J. H. and Keller, C. H. (1996). Motor control of the jamming avoidance response of *Apteronotus leptorhynchus*: evolutionary changes of a behavior and its neural substrate. *J. Comp. Physiol. A* **179**, 653-674.
- Heinsohn, R., Legge, S. and Endler, J. A. (2005). Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science* **309**, 617-619.
- Hopkins, C. D. (1974a). Electric communication in fish. *Am. Sci.* **62**, 426-437.
- Hopkins, C. D. (1974b). Electric communication in the reproductive behavior of *Sternopygus macrurus* (Gymnotoidei). *Psychol.* **35**, 518-535.
- Hopkins, C. D. (1974c). Electric communication: functions in the social behavior of *Eigenmannia virescens*. *Behaviour* **50**, 270-305.
- Hopkins, C. D. (1988). Neuroethology of electric communication. *Annu. Rev. Neurosci.* **11**, 497-535.
- Hopkins, C. D. (1999). Design features for electric communication. *J. Exp. Biol.* **202**, 1217-1228.
- Irwin, D. E. (2000). Song variation in an avian ring species. *Evolution* **54**, 998-1010.
- Kolodziejcki, J. A., Nelson, B. S. and Smith, G. T. (2005). Sex and species differences in neuromodulatory input to a premotor nucleus: a comparative study of substance P and communication behavior in weakly electric fish. *J. Neurobiol.* **62**, 299-315.
- Kramer, B., Kirschbaum, F. and Markl, H. (1980). Species specificity of electric organ discharges in a sympatric group of gymnotoid fish from Manaus (Amazonas). In *Sensory Physiology of Aquatic Lower Vertebrates* (ed. T. Szabó and G. Czéh), pp. 195-219. Budapest: Akadémiai Kiadó.
- Larimer, J. L. and MacDonald, J. A. (1968). Sensory feedback from electroreceptors to electromotor pacemaker centers in gymnotids. *Am. J. Physiol.* **214**, 1253-1261.
- Mago-Leccia, F., Lundberg, J. G. and Baskin, J. N. (1985). Systematics of the South American freshwater fish genus *Adontosternarchus* (Gymnotiformes, Apteronotidae). *Contrib. Sci. Los Angeles* **358**, 1-19.
- Meyer, J. H. (1983). Steroid influences upon the discharge frequencies of a weakly electric fish. *J. Comp. Physiol. A* **153**, 29-37.
- Meyer, J. H., Leong, M. and Keller, C. H. (1987). Hormone-induced and maturational changes in electric organ discharges and electroreceptor tuning in the weakly electric fish *Apteronotus*. *J. Comp. Physiol. A* **160**, 385-394.
- Mills, A. and Zakon, H. H. (1987). Coordination of EOD frequency and pulse duration in a weakly electric wave fish: the influence of androgens. *J. Comp. Physiol. A* **161**, 417-430.
- Moller, P. (1995). *Electric Fishes: History and Behavior*. London: Chapman & Hall.
- Price, J. J. and Lanyon, S. M. (2004). Patterns of song evolution and sexual selection in the oropendolas and caciques. *Behav. Ecol.* **15**, 485-497.
- Serrano-Fernandez, P. (2003). Gradual frequency rises in interacting black ghost knifefish, *Apteronotus albifrons*. *J. Comp. Physiol. A* **189**, 685-692.
- Smith, G. T. (1999). Ionic currents that contribute to a sexually dimorphic communication signal in weakly electric fish. *J. Comp. Physiol. A* **185**, 379-387.
- Smith, G. T. and Zakon, H. H. (2000). Pharmacological characterization of ionic currents that regulate the pacemaker rhythm in a weakly electric fish. *J. Neurobiol.* **42**, 270-286.
- Stoddard, P. K. (1999). Predation enhances complexity in the evolution of electric fish signals. *Nature* **400**, 254-256.
- Stoddard, P. K. (2006). Plasticity of the electric organ discharge waveform: contexts, mechanisms, and implications for electrocommunication. In *Communication in Fishes*. Vol. 3 (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 623-646. Enfield, NH: Science Publisher.
- Zakon, H. H. and Smith, G. T. (2002). Weakly electric fish: behavior, neurobiology, and neuroendocrinology. In *Hormones, Brain, and Behavior*. Vol. 2 (ed. D. Pfaff, A. Arnold, A. Etgen, S. Fahrbach, R. Moss and R. Rubin), pp. 349-374. New York: Academic Press.
- Zakon, H. H., Thomas, P. and Yan, H.-Y. (1991). Electric organ discharge frequency and plasma sex steroid levels during gonadal recrudescence in a natural population of the weakly electric fish *Sternopygus macrurus*. *J. Comp. Physiol. A* **169**, 493-499.
- Zupanc, G. K. H. and Maler, L. (1993). Evoked chirping in the weakly electric fish *Apteronotus leptorhynchus* – a quantitative biophysical analysis. *Can. J. Zool.* **71**, 2301-2310.