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Diversity of sexual dimorphism in electrocommunication signals and its androgen regulation in a genus of electric fish, *Apteronotus*

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Abstract Gymnotiform electric fish emit an electric organ discharge that, in several species, is sexually dimorphic and functions in gender recognition. In addition, some species produce frequency modulations of the electric organ discharge, known as chirps, that are displayed during aggression and courtship. We report that two congeneric species (*Apteronotus leptorhynchus* and *A. albifrons*) differ in the expression of sexual dimorphism in these signals. In *A. leptorhynchus*, males chirp more than females, but in *A. albifrons* chirping is monomorphic. The gonadosomatic index and plasma levels of 11-ketotestosterone were equivalent in both species, suggesting that they were in similar reproductive condition. Corresponding to this difference in dimorphism, *A. leptorhynchus* increases chirping in response to androgens, but chirping in *A. albifrons* is insensitive to implants of testosterone, dihydrotestosterone or 11-ketotestosterone. Species also differ in the sexual dimorphism and androgen sensitivity of electric organ discharge frequency. In *A. leptorhynchus*, males discharge at higher frequencies than females, and androgens increase electric organ discharge frequency. In *A. albifrons*, males discharge at lower frequencies than females, and androgens decrease electric organ discharge frequency. Thus, in both chirping and electric organ discharge frequency, evolutionary changes in the presence or direction of sexual dimorphism have been accompanied and perhaps caused by changes in the androgen regulation of the electric organ discharge.

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Abbreviations *EOD* electric organ discharge · *11KT* 11-ketotestosterone · *DHT* dihydrotestosterone · *T* testosterone · *GSI* gonadosomatic index

Introduction

Virtually all sexual species exhibit some form of sexually dimorphic behavior. Yet even among closely related species, the magnitude and direction of sexual dimorphism can vary substantially. While there are numerous examples of such diversification in the expression of dimorphic behavior (Andersson 1994), the mechanistic basis of evolutionary reversals and loss of sexually dimorphic behavior has seldom been examined. Here we report that the species differences in the sexually dimorphic electrocommunication behavior in two congeneric gymnotiform electric fish may be attributable in part to species differences in the response to androgens.

South American electric fish (Order Gymnotiformes) produce electric signals from a specialized organ in their tail. In wave-type species, the electric organ discharge (EOD) has quasi-sinusoidal wave form and is emitted continuously. Each species has its own characteristic range of discharge frequencies, and within many species, males and females differ in their EOD frequency. Weakly electric fish use the EOD both for locating objects around them and for social communication. In several species, individuals can use the sexually dimorphic EOD frequency to recognize the gender of a conspecific. In *Apteronotus albifrons*, as in most other species examined, females discharge at higher frequencies than males. However, in the congeneric species *A. leptorhynchus*, males have a higher discharge frequency than females (Hagedorn and Heiligenberg 1985; Meyer et al. 1987). This species difference indicates there has been an evolutionary reversal in the direction of sexually dimorphic behavior within *Apteronotus*.

Several studies have shown that androgens modulate the EOD frequency, and thereby participate in the generation of sexually dimorphic behavior. Laboratory experiments show that androgen treatment generally causes the EOD frequency to shift in the male-typical direction, and various forms of androgen [testosterone (T), dihydryotestosterone (DHT), 11-ketotestosterone (11KT)] differ in their potency and effect (Meyer 1983; Meyer et al. 1987; Mills and Zakon 1991; Schaefer and Zakon 1996). Also, in one species (*Sternopygus macrurus*), both the pre-pacemaker nucleus in the brain and the electrocytes of the electric organ contain androgen receptors (Gustavson et al. 1994), indicating that cells that generate the signal are influenced directly by endogenous androgens. Thus, among species that differ in the direction of sexually dimorphic EOD frequency, androgen regulation of the EOD may have been a locus of evolutionary change.

In addition to the continuous basal EOD frequency, several wave-type species exhibit modulations of their EOD. One such modulation, the chirp, is a rapid, transient increase in the discharge frequency (Larimer and Macdonald 1968; Hopkins 1974). The exact function of chirps is not entirely clear, but they have been observed during social interactions, particularly during courtship and aggression (Hagedorn and Heiligenberg 1985). Chirps can be evoked reliably in *A. leptorhynchus* by presenting a fish with an electrical stimulus at a frequency close to its own EOD frequency. Such evoked chirping is both sexually dimorphic (Dye 1987; Zupanc and Maler 1994) and androgen dependent (Dulka and Maler 1994). Males are much more likely to chirp than females, but females increase their chirping in response to androgen treatment. Although chirps are likely an important component of sexual communication in electric fish, few species have been examined for the ability to chirp or for sexual dimorphism in chirping.

Our aim in this study was to examine whether *A. albifrons* and *A. leptorhynchus* differ in sexually dimorphic electrocommunication behavior, and if so, whether they are differentially responsive to androgens. In particular, we directly compared the two species in their expression of sexually dimorphic chirping behavior, and tested the effect of androgens on EOD frequency and chirping of *A. albifrons*, and compared it to the response of *A. leptorhynchus* reported previously (Dulka and Maler 1994).

Materials and methods

Experiment 1 was designed to identify sexual differences in chirping and EOD frequency in both *A. albifrons* and *A. leptorhynchus*. Intact *A. albifrons* (nine males, seven females) and *A. leptorhynchus* (ten males, six females) were tested for chirping. Blood was then collected from the vertebral sinus for measurement of plasma steroid concentrations and the gonads and body were weighed for measurement of gonado-somatic index (GSI, gonad mass × 100/body mass).

Experiment 2 assessed the effect of androgens on chirping and EOD frequency in *A. albifrons*. Twenty-four *A. albifrons* were gonadectomized and allowed to recover for >4 weeks. All fish were then bled to confirm that gonadectomy had indeed lowered plasma androgen concentrations. All fish were divided into four equivalent groups, with equal distributions of sex ratio (three males and three females/group), standard length (SL, in mm), EOD frequency (in Hz) and chirping frequency (chirps min^{-1} at –35 dB). Control fish (SL = 21.2 ± 2.5; EOD frequency = 1057 ± 25) received empty capsules. Among experimental fish, one group (SL = 19.5 ± 1.9; EOD = 1059 ± 20) received capsules containing T, another group (SL = 20.3 ± 1.6; EOD = 1049 ± 18) received capsules of DHT, and the last group (SL = 22.3 ± 2.3; EOD = 1052 ± 33) received capsules of 11KT. Chirping was measured 1–2 days before implantation, 13–14 days after implantation and 42–43 days after implantation. EOD frequency was measured every 2–7 days beginning 2 days before implantation until 42 days following implantation. At 44 days post-implantation fish were bled again to assess experimental levels of androgens.

Animals and housing conditions

A. albifrons and *A. leptorhynchus* were obtained from commercial dealers and housed in individual tanks that were part of a circulating 400 gallon system. Fish were fed every 2–3 days; *A. albifrons* received a combination of frozen brine shrimp, earthworms and commercial fish food (Food Sticks for Carnivores, Hikari), and *A. leptorhynchus* received only brine shrimp. Water conditions were controlled at a constant temperature (28.2 ± 0.2 °C), pH (6.5) and conductivity (850 μS), and photoperiod was kept at 12L:12D. Fish acclimated to these conditions for >3 weeks prior to each experiment.

Chirp testing apparatus

We used a testing apparatus similar to that used by Dulka and Maler (1994). Fish were tested in a clear Plexiglass tube (8 cm diameter × 24 cm long, with two 5 × 7 cm sections cut from the mid-point of each side. Each of these windows and one end of the tube were covered with nylon mesh; the other end was fitted with a removable door. Stimuli were presented to the fish through two carbon electrodes attached to the apparatus at the mid-point of the windows and positioned orthogonal to the axis of the fish's body.

Sinusoidal stimulus signals were generated by a pulse generator (WAVETEK) and passed through an attenuator which allowed us to vary the signal amplitude. The signal was then passed through a transformer to filter d.c. offset, and delivered to the fish through the carbon electrodes. We varied stimulus amplitude by two orders of magnitude, presenting stimuli at –55, –45, –35, –25, –15 dB. The electrical field created by these stimuli varied in intensity from the center to the periphery of the Plexiglass tube. The field intensities at each stimulus amplitude were (from center to periphery, in mV cm^{-1}): 0.06–0.09 (–55 dB), 0.18–0.28 (–45 dB), 0.52–0.88 -(–35 dB), 1.60–2.60 (–25 dB) and 5.10–8.20 (–15 dB). The precise frequency of the stimulus was measured by a frequency counter (FLUKE 7260A).

The fish's EOD was recorded through Ag-AgCl electrodes attached to the ends of the tube (parallel to the fish's body axis). The signal was amplified and monitored continuously on an oscilloscope (TEKTRONICS) and frequency counter and recorded on video tape.

Measurement of chirping behavior and EOD frequency

For measuring evoked chirping, fish were removed individually from their home aquaria and placed in the testing apparatus, which was submerged in a 20-l testing aquarium. The water in the test aquarium was circulated, and the temperature (28.2 °C) and conductivity (850 μS) were kept identical to those of the fish's home tank. Fish were allowed to acclimate to the test environment for at least 30 min before testing.

In intial studies, the stimulus signal was adjusted to 3–5 Hz below or 3–5 Hz above the fish's EOD. We found no differences in response to these two stimuli in either sex of either species ($P > 0.05$). To be consistent with previous studies of chirping, we chose to present only stimuli 3–5 Hz below the fish's EOD for all subsequent experiments. Stimuli were presented once for 60 s at each of five amplitudes (–55 to –15 dB), with a pause of at least 60 s between each stimulus presentation. Changes in stimulus strength always proceeded from low to high amplitude. After all stimuli were presented, fish were returned to their home tank. No fish was tested more than once in a 2 week period.

Chirping behavior was quantified in two ways: the proportion of individuals in each treatment group (species, gender, hormonal status) that exhibited chirping (propensity to chirp) and the number of chirps during 1 min of stimulus (chirp frequency).

To measure changes in EOD frequency, we recorded the EOD through two bare wires mounted on a Plexiglas rod which was held within 1 cm of the fish. The signal was fed into a Grass P15 amplifier and subsequently analyzed with a FLUKE voltmeter in the frequency counter mode. To avoid any possible confounding diel variation in EOD, all measurements were taken at the same time of day (1200–1400 hours). All EOD measurements were standarized to a temperature of 28.0 °C using a Q_{10} of 1.5 (Enger and Szabo 1968)

Gonadectomy and hormone implantation

Surgical gonadectomy was similar to that reported previously (Dunlap et al. 1997). Fish were anesthetized in 0.05% 2-phenoxyethanol (Sigma, P-1126). Gonads and accessory ducts were removed through a ~1-cm incision in the posterior ventrolateral body wall. The incision was closed with four to five sutures (5.0 surgical silk), the wound was treated with antibiotic, and the fish was allowed to recover in its home tank.

To manipulate the hormonal condition of each fish, we implanted fish with capsules filled with one of the three androgens: T (Sigma T-8390), DHT (Sigma A-8380), and 11KT (Sigma K-8250). Capsules consisted of silastic tubes (0.64 mm inside diameter × 1.20 mm outside diameter; Dow-Corning #602–155) filled with steroids and sealed at each end with silastic adhesive. Capsules varied in length depending on the size of the fish (~2 mm/10 g body wt). Each capsule contained ~1–2 mg steroid, resulting in a total dosage ~100 µg g body wt^{-1}. Control fish received an empty capsule. To implant the capsules, we anesthetized the fish as above, punctured a small hole in the lateral body wall using an 18-gauge needle, and inserted the capsules into the anterior peritoneal cavity.

Blood collection and hormone analysis

Fish were anesthetized until movement of the ventral fin stopped. A heparinized 25-gauge needle was inserted in to the vertebral sinus, and 150–300 µl of blood was drawn into a heparinized syringe. The blood was stored on ice for 1–3 h until centrifugation. Plasma was stored at –20°C until hormone analysis.

Plasma 11KT and T levels were determined by radioimmunoassay in the laboratory of P. Thomas, University of Texas Marine Science Institute. This assay was previously validated for another gymnotiform fish, *Sternopygus*, in the Thomas laboratory (Zakon et al. 1991). Steroids were extracted from 50–100 µl plasma with a 70:30 mixture of hexane and ethyl acetate. The aqueous portion was removed, the solvent layer was evaporated with N_2, and the hormone residue was reconstituted in phosphate buffer. Each sample was incubated 2–3 h at room temperature with the appropriate antiserum and tritiated steroid. The unbound fraction was removed by charcoal (4°C) and centrifugation, and the bound fraction was counted in a scintillation counter. All samples were run in a single assay.

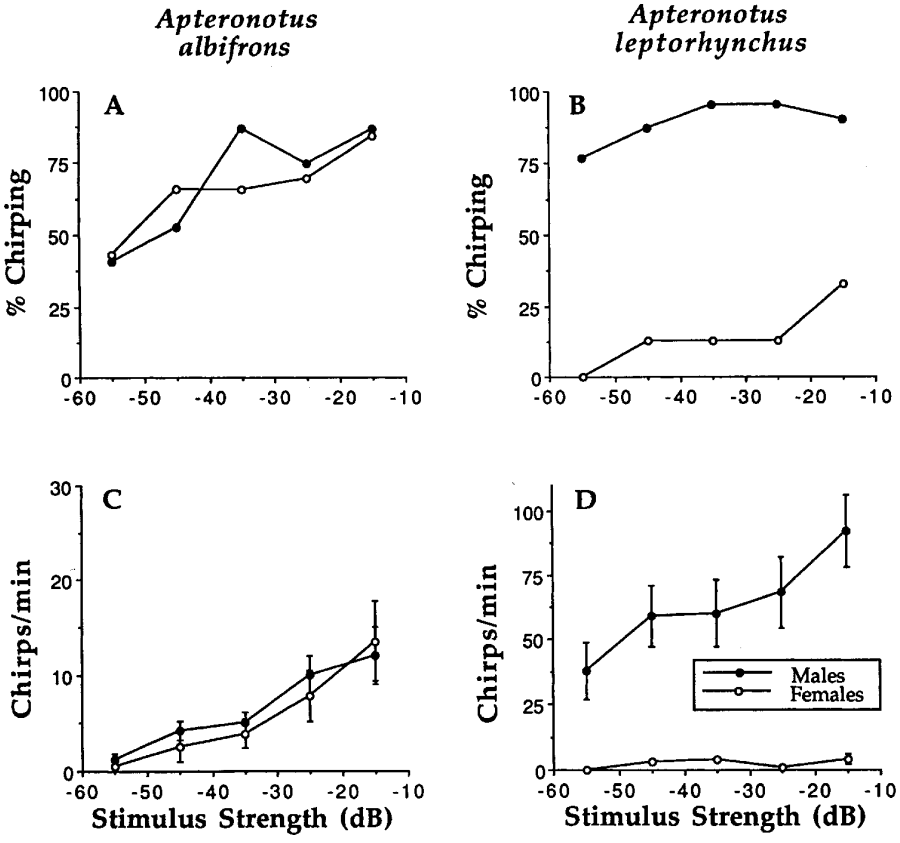


Fig. 1A–D Chirp propensity (% fish chirping; **A, B**) and frequency (chirps per minute; **C, D**) in male (*solid circles*) and female (open circles) *Apteronotus albifrons* (**A, C**) and *A. leptorhynchus* (**B, D**). Sinusoidal electrical stimuli were presented at five different strengths. Chirping is monomorphic in *A. albifrons* and highly dimorphic in *A. leptorhynchus*. Note the difference in scale of the ordinant in **C** and **D**: male *A. leptorhynchus* chirp with much greater frequency than male *A. albifrons*

Based on previous studies, the T antibody (generated in the Thomas laboratory from a conjugated steroid obtained from Cambridge Medical Diagnostics) reacts 28.0% with DHT and 17.0% with 11KT, and the 11KT antibody (Helix Biotech) cross-reacts 17% with DHT and 9% with T (Zakon et al. 1990). Because the antisera were not completely specific and steroids were not separated chromatographically, the values we present for circulating steroid concentrations should only be considered approximations; true values are probably slightly lower than those reported.

Statistics

To identify species and sexual differences in EOD frequency, chirp frequency, body size, GSI and androgen levels, we used a two-way ANOVA with species and gender as independent variables. A significant interaction term (species × gender) indicated a species difference in sexual dimorphism. The effects of androgen treatment on EOD frequency and chirp frequency were analyzed using repeated-measures ANOVA with time as the repeated measure. This test compares changes in individuals through time, and thus detects significant changes between pre- and post-implantation values. A significant interaction term (time × treatment) indicates that treatment groups changed differently over time. P-values < 0.05 were considered significant.

Results

Experiment 1: sexual dimorphism in *A. albifrons* and *A. leptorhynchus*

Chirping

A. albifrons did not exhibit sexual dimorphism in either the propensity (Fig. 1; $F = 1.2$, $df = 1$, $P > 0.05$) or frequency of chirping ($F = 1.4$, $df = 1$, $P > 0.05$). Regardless of gender, a minimum of 45% (at –55 dB) and a maximum of 87% (at –15 dB) of fish chirped. Chirp frequency in *A. albifrons* was also monomorphic, ranging from 1.6 ± 0.5 (–55 dB) to 6.6 ± 2.2 chirps min^{-1} (–15 dB) (Fig. 1).

Replicating previous results (Dye 1987; Zupanc and Maler 1994), we found that chirping in intact *A. leptorhynchus* is sexually dimorphic. At all stimulus strengths, males chirped with propensity approximately three to five times greater than females (Fig. 1). Chirp frequency (chirps per minute) was also sexually dimorphic: males ranged in chirp frequency from 37.5 ± 11.3 (–55 dB) to 94.6 ± 11.1 (–15 dB), and females ranged from 0 (–55 dB) to 3.1 ± 0.7 (–15 dB).

At all stimulus strengths, *A. albifrons* of both sexes were slightly less likely to exhibit chirping than male *A. leptorhynchus* and much more likely to chirp than female *A. leptorhynchus*. Both male and female *A. albifrons* chirped at lower rates than male *A. leptorhynchus* and slightly more than female *A. leptorhynchus*.

EOD frequency

EOD frequency was sexually dimorphic in *A. albifrons* ($F = 3.3$, $P < 0.05$), with females discharging at a higher frequency than males (Table 1). As reported by other researchers, EOD frequency was also sexually dimorphic in *A. leptorhynchus* ($F = 12.4$, $df = 1$, $P < 0.005$) in a direction opposite to that of *A. albifrons*.

Body size and relative gonad size

Among the fish we tested, there was a significant sexual difference in body size in both *A. albifrons* ($F = 4.1$, $df = 1$, $P < 0.05$) and *A. leptorhynchus* (Table 1; $F = 8.4$, $df = 1$, $P < 0.001$). The species × gender interaction term was also significant ($F = 3.8$, $df = 1$, $P < 0.05$), indicating that *A. leptorhynchus* is significantly more dimorphic than *A. albifrons*. There was no difference between *A. albifrons* and *A. leptorhynchus* in the GSI of either males or females ($F = 1.7$, $df = 1$, $P > 0.05$).

Plasma androgen concentrations.

Plasma 11KT levels were about eight to nine times higher in males than in females in both *A. albifrons* and *A. leptorhynchus* (Table 1; $F = 7.4$, $df = 1$, $P < 0.001$). There was no significant effect of species ($F = 1.1$, $df = 1$, $P > 0.05$) or species × gender interaction ($F = 0.97$, $df = 1$, $P > 0.05$). Plasma T levels were similar in males and females in both species and there was no significant species difference ($F = 0.82$, $df = 1$, $P > 0.05$). In males of both species, T concentrations were higher than 11KT concentrations ($F = 3.2$, $df = 1$, $P < 0.05$); T also showed considerably more inter-individual variability than 11KT.

Table 1 Sexual dimorphism in body size, EOD frequency, plasma androgen leves and gonadosomatic index (gonad mass × 100/body mass) in intact *Apteronotus albifrons* and *A. leptorhynchus*. Data are presented as mean ± SE

	Apteronotus albifrons		*Apteronotus leptorhynchus*	
	Male ($n = 9$)	Female ($n = 7$)	Male ($n = 10$)	Female ($n = 6$)
Body length (mm)	18.6 ± 0.8	16.0 ± 0.3	16.8 ± 0.6	13.1 ± 0.2
EOD frequency (Hz)	1057 ± 18	1116 ± 24	903 ± 22	772 ± 15
11-KT (ng ml^{-1})	6.12 ± 1.22	0.82 ± 0.12	5.33 ± 1.74	0.89 ± 0.25
T (ng ml^{-1})	8.45 ± 3.2	14.21 ± 4.1	10.6 ± 2.41	12.2 ± 3.11
Gonadosomatic index	0.096 ± 0.006	1.867 ± 0.281	0.105 ± 0.007	2.612 ± 0.903

Experiment 2: effects of androgen on electrocommunication signals in *A. albifrons*

Chirping

An implant of T, DHT or 11KT failed to affect chirping behavior in gonadectomized *A. albifrons*. At 2 and 6 weeks following steroid treatment, neither chirping propensity (Fig. 2) nor chirp frequency (Fig. 3) were different from pre-implantation values in any treatment group: there was no significant effect of treatment ($F = 1.4$, $df = 4$, $P > 0.05$), time (the repeated measure; $F = 2.1$, $df = 1$, $P > 0.05$) or treatment × time interaction ($F = 1.3$, $df = 1$, $P > 0.05$).

EOD frequency

At doses that were ineffectual in modifying chirping, DHT and 11KT decreased EOD frequency in *A. albifrons* (Fig. 4). Implantation of an empty capsule (control) or T-filled capsule had no effect on EOD frequency ($F = 0.73$, $P > 0.05$). Mean change in EOD frequency decreased in the DHT and 11KT-treated groups beginning 4 days after implantation, with the decrease becoming statistically significant 22 days after implantation. For the DHT and 11KT groups, this represented a percentage change of –5.2 ± 1.2 and –2.6 ± 1.5, respectively.

Plasma androgen concentrations

Plasma 11KT and T levels were lower in gonadectomized males and females compared to intact fish (Tables 1, 2), indicating that gonadectomy effectively lowered circulating androgen levels. Control implants had no effect on androgen concentrations. Implantation with 11KT capsules increased circulating levels to ~1.7 times the mean level found in intact males and equivalent to the highest values in intact males. T and DHT treatments also apparently elevated 11KT values (compared to control treatment, Table 2). However, plasma 11KT levels of these other androgens were much lower in these two androgen treatment groups than in the 11KT group and are likely attributable to their cross-reactivity with the 11KT antisera.

Mean plasma T levels in T-treated fish were equivalent to those of intact males and females. Plasma T was also elevated in 11KT-treated fish (compared to controls), but again, this may be an artefact of cross-reactivity of the T antibody with 11KT.

Discussion

We found that male *A. albifrons* discharge at lower frequencies than females (Table 1), but that males and females do not differ in chirping responses to electrical stimuli (Fig. 1). This contrasts markedly with the closely related *A. leptorhynchus*, in which males discharge at higher frequencies than females and males show greater

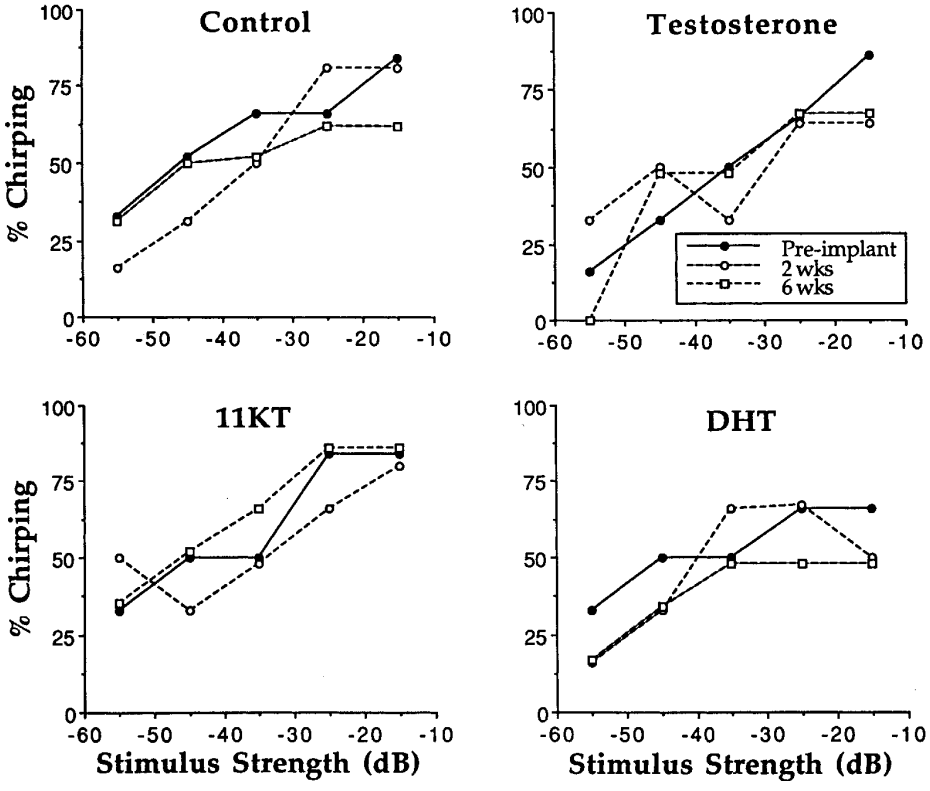


Fig. 2. Chirp propensity in gonadectomized *A. albifrons* implanted with empty capsules (control) or capsules filled with androgens testosterone, 11-ketotestosterone (11KT) or dihydrotestosterone (DHT). Fish were tested 2 days before implantation (*solid circle*, *solid line*), 2 weeks after implantation (*open circle*, *dashed line*) and 6 weeks after implantation (*open square*, *dashed line*). Androgen treatment had no effect on chirp propensity

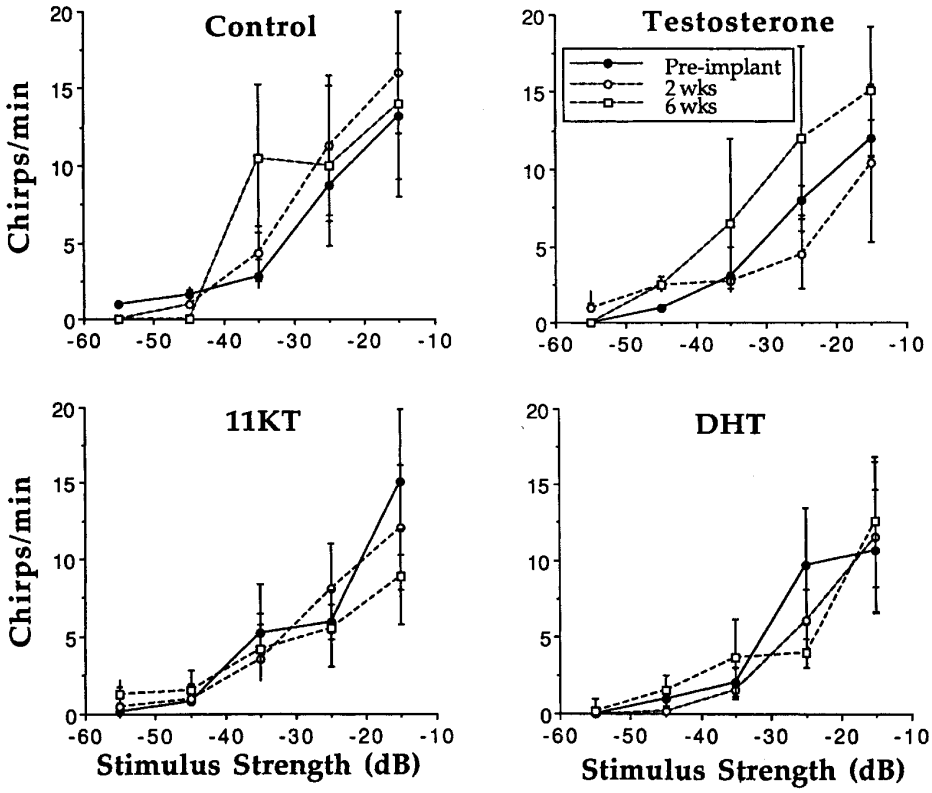


Fig. 3 Chirp frequency in gonadectomized *A. albifrons* implanted with empty capsules (control) or capsules filled with androgens (testosterone, 11KT or DHT). Androgen treatment had no effect on chirp frequency. Data are presented as mean ± SE. See Fig. 2 for symbol definitions

chirping responses than females. These species differences in behavior are parallel with, and are perhaps caused by, differential responses to androgens. In *A. albifrons*, androgen treatment (11KT and DHT) lowers EOD frequency (Fig. 4) but has no effect on chirping behavior (Figs. 2, 3). In *A. leptorhynchus*, androgen (11KT) raises EOD frequency (Meyer et al. 1987; Schaefer and Zakon 1996) and increases the propensity and frequency of evoked chirping (J. Dulka, personal communcation).

Combining these results with other hormonal and molecular systematic studies of gymnotiforms, we hypothesize that there was an evolutionary reversal in sexual dimorphism in EOD frequency and its androgen dependence in the lineage that includes *A. leptorhynchus*.

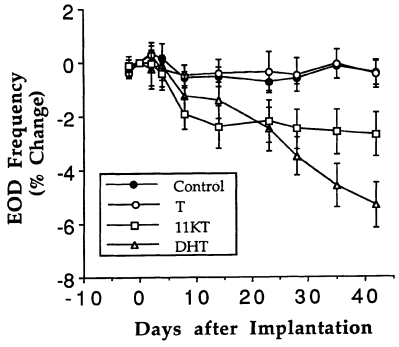


Fig. 4 Change in EOD frequency in gonadectomized *A. albifrons* implanted with empty capsules (control, *closed circle*) or capsules filled with either testosterone (T) (*open circle*), 11KT (*open square*) or DHT (*open triangle*). Data are presented as mean ± SE

In addition, sexual dimorphism in chirping and its androgen sensitivity was apparently lost in the lineage that includes *A. albifrons* (Fig. 5).

Diversity of sexual dimorphism within *Apteronotus*

A. albifrons shows relatively little sexual dimorphism in both electrocommunication signals and morphology. Male and females chirp with equal propensity and frequency in response to sinusoidal electric stimuli (Fig. 1). Sexual dimorphism in evoked chirping could conceivably result from sexual differences in the sensory perception of the stimulus or in the subsequent electromotor output. The fact that males and females chirped equivalently at all stimulus strengths supports the notion that both sensory perception and motor response are monomorphic.

Under identical testing circumstances, *A. leptorhynchus* exhibited sexual differences in both chirp propensity and frequency at all stimulus strengths. Species differences in sexual dimorphism were attributable to species differences in the response of both males and females: male *A. albifron*s chirp less than male *A. leptorhynchus*, and female *A. albifrons* chirp more than female *A. leptorhynchus*.

We did not analyze chirp structure quantitatively, but the evoked chirps of these species appeared qualitatively similar to each other and to chirps evoked by male conspecifics. For both species, chirps were frequently accompanied by attacks towards the stimulus electrodes,

Table 2 EOD frequency and plasma androgen levels in gonadectomized *Apteronotus albifrons* 2 days before and 6 weeks after implantation with androgens. Controls received an empty implant. Data are presented as mean ± SE. Numbers in parentheses are sample sizes. NA = not available

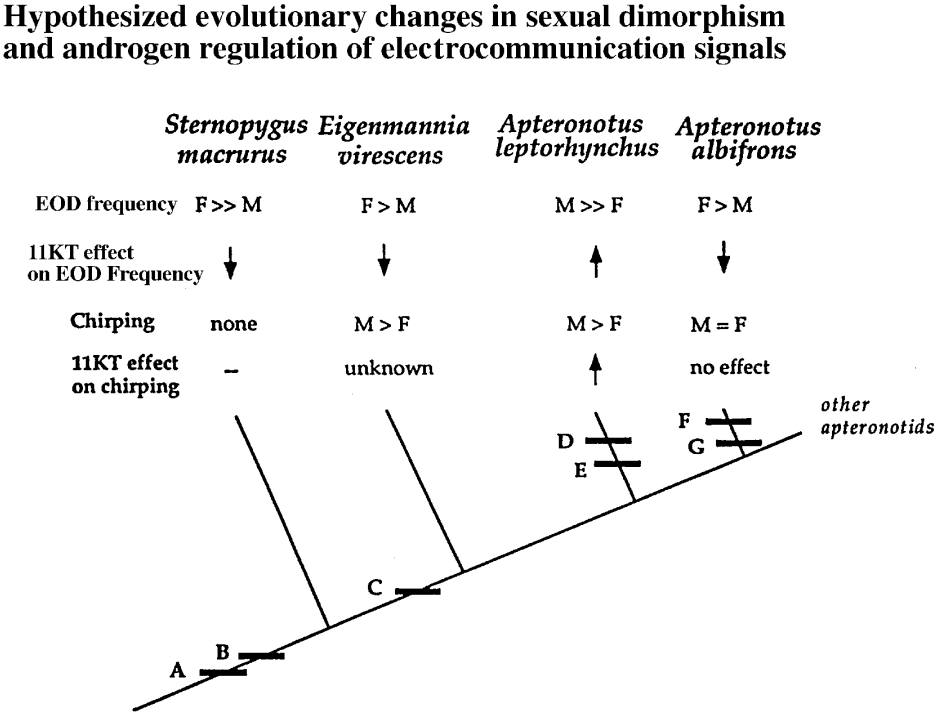
	Pre-implantation		Hormone implanted			
	Males	Females	Control	11-KT	T	DHT
EOD frequency (Hz)	1055 ± 34 (12)	1063 ± 36 (12)	1051 ± 24 (6)	1015 ± 15 (6)	1048 ± 20 (6)	986 ± 22 (6)
11KT (ng ml^{-1})	0.38 ± 0.24 (11)	0.23 ± 0.22 (10)	0.28 ± 0.16 (5)	8.91 ± 2.21 (5)	1.03 ± 0.65 (4)	0.88 ± 0.42 (4)
T (ng ml^{-1})	0.51 ± 0.34 (10)	0.45 ± 0.29 (9)	0.46 ± 0.32 (6)	1.01 ± 0.87 (4)	11.92 ± 3.31 (5)	NA

and consequently, we interpret these chirps as similar to those displayed in an aggressive context rather than those during courtship.

EOD frequency was statistically different in male and female *A. albifrons*, but there was considerable overlap in the frequency distributions. It is possible that sexual dimorphism in EOD frequency may not have been fully expressed in captivity (see below). However, if field-active fish show a similar degree of sexual dimorphism as those in the laboratory, EOD frequency could not be used as the sole signal for gender identification. EOD frequency may nonetheless be important in augmenting other communication signals for gender recognition (e.g., olfactory or visual cues) or in signaling particular aspects of mate quality (e.g., dominance rank, size, etc.). In contrast to *A. albifrons*, *A. leptorhynchus* showed a strong sexual dimorphism in EOD frequency with very little overlap in the frequency distributions of males and females. Thus, EOD frequency in this species could be used as a reliable indicator of gender identity.

In addition to showing relatively less dimorphism in electrocommunication signals, *A. albifrons* is less dimorphic in morphology than *A. leptorhynchus*. In *A. albifrons*, mean male body length was 16% greater than that of females; in *A. leptorhycnius*, males were on average 28% longer than females. In other studies, we have found that male *A. leptorhynchus* were as much as 50% longer and 100% heavier than females. Male and

Fig. 5 Proposed hypothesis for the evolution of sexual dimorphism in and androgen effects on electric organ discharge (EOD) frequency and chirping in four species of wave-type gymnotiform electric fish. Data are super-imposed on a cladogram derived from the molecular phylogeny of Alves-Gomes et al. (1995). *Letters* on cladogram indicate the hypothesized series of evolutionary changes that account for current diversity of sexual dimorphism and its androgen regulation in these species. In the ancestral condition, males discharged at lower frequencies than females (*A*) and 11KT lowered EOD frequncy (*B*). Chirping evolved after the divergence of *Sternopygus*, with males chirping more than females (*C*). Sexual dimorphism in EOD frequency (*D*) and the effect of 11KT (*E*) reversed in the lineage containing *A. leptorhynchus*. Sexual dimorphism in chirping (*F*) and its sensitivity to 11KT (*G*) was lost in the lineage containing *A. albifrons*. *F* and *G* could also lie on the branch preceding the divergence of *A. albifrons*. This figure depicts the most parsimonious interpretation of species studied thus far; information from future studies of other species could modify this interpretation



female *A. leptorhynchus* also differ in body shape, with males having a relatively elongate snout (Hagedorn and Heiligenberg 1985). We found no obvious dimorphism in the body shape of *A. albifrons*.

We recognize that comparative studies of sexual dimorphism on captive, imported species can be difficult to interpret because one does not know whether animals entered the laboratory in equivalent reproductive condition and/or whether captivity affects the expression of sexual dimorphism in species differentially. For example, in our study, species may have differed in sexually dimorphic chirping because only *A. leptorhynchus*, and not *A. albifrons*, were in full reproductive condition. We partially addressed this issue by directly comparing the plasma steroid levels and GSI of both species. We found no difference in either measure of reproductive state (Table 1). Nevertheless, it is possible that *A. albifrons* show greater sexual dimorphism in the field, where steroid levels and GSI may be higher than those measured in the laboratory. Direct comparisons of the physiology and behavior of naturally breeding fish are obviously necessary to substantiate our laboratory results.

Androgen regulation of electrocommunication signals in *Apteronotus albifrons*

Treatment of gonadectomized *A. albifrons* with any of three androgens failed to alter chirping behavior (Figs. 2, 3). The dosage we used elevated plasma 11KT and T levels to those found in intact males (Tables 1, 2), and thus our treatment was successful in generating physiologically relevant plasma concentrations. Our dosages of 11KT and DHT were also effective in modifying EOD frequency (Fig. 4), indicating that the exogenous androgens were capable of influencing neurons in the electrocommunication system. The apparent androgen independence of chirping is corroborated by an analysis of individual variability of androgens and behavior: among both intact and androgen-treated fish, there was no correlation between the degree of chirping and plasma androgen levels. Nevertheless, it is possible that androgen levels higher than those exhibited in our laboratory may induce chirping in *A. albifrons*. Again, measurement of maximal androgen concentrations in naturally breeding fish is necessary to evaluate this possibility.

We did not test the effect of androgens on chirping in *A. leptorhynchus*, but Dulka reported that implants of T, DHT (Dulka et al. 1995) or 11KT (J. Dulka, personal communication) similar in size to those we used are effective in increasing the propensity and frequency of chirping in female *A. leptorhynchus*. (However, T-treated females in these studies did not chirp with as high propensity or frequency as males in our study, even though T treatment resulted in circulating levels similar to our intact males). In other studies, chirp frequency during staged encounters between intact male *A. leptorhynchus* correlates with endogenous plasma 11KT levels (K. Dunlap, unpublished observations). All of these studies indicate that chirping behavior of *A. leptorhynchus* is responsive to androgens at concentrations that are ineffective in modifying the chirping behavior of *A. albifrons*. Thus, even if androgens affect chirping in *A. albifrons* at higher dosages, our results indicate chirping in *A. albifrons* is at least less sensitive to androgens than chirping in *A. leptorhynchus*.

11KT, the androgen that most typically regulates male teleost behavior, decreases EOD frequency in *A. albifrons*, as one would expect given that males have lower EOD frequencies than those of females. Interestingly, 11KT has the reverse effect in *A. leptorhynchus*, but it nevertheless masculinizes the signal because males normally discharge at higher frequencies than females in this species. Thus, two species with opposite directions in sexual dimorphism have opposite responses to the principal behaviorally potent androgen in teleosts.

A. albifrons and *A. leptorhynchus* also differ in their responses to other androgens, but in ways that are presently less explicable. In *A. leptorhynchus*, T decreases the EOD frequency, shifting the EOD in a more female-typical direction. This effect mimics the response to estradiol and can be blocked with an aromatase inhibitor, indicating that T likely achieves is feminizing effect via conversion to estradiol (M. Zucker, unpublished data). However, in *A. albifrons*, T had no effect on EOD frequency (Fig. 4). This is particularly curious since three major T metabolites, DHT, 11KT (Fig. 4) and estradiol (K. Dunlap, unpublished data), all lower EOD frequency. T may have been metabolized to some other product that does not bind to androgen receptors. The two species also differ in their response to DHT, which lowered EOD frequency in *A. albifrons*, but had no effect on EOD frequency in *A. leptorhynchus*.

The diversity of androgen effects on the EOD frequency of these two species suggest that there have been substantial evolutionary changes in androgen metabolism, androgen receptor distribution and/or androgen receptor binding within *Apteronotus*. We have begun investigating whether different androgens exert diverse effects in *Apteronotus* by binding to multiple androgen receptors with different affinities. At least two forms of androgen receptors have been isolated in teleosts, and these receptors differentially bind several androgens (Pasmanik and Callard 1988; Fitzpatrick et al. 1994; T. Sperry, personal communication). The presence of multiple androgen receptor forms in the brain of *Apteronotus* may help explain the unpredicted effects of DHT and T on their electric signals.

Although we have focused on the masculinizing effects of androgens, it is important to note that sexual dimorphism in EOD frequency can be generated by seasonal changes in females as well as males. In nonbreeding condition, males and females typically have overlapping, intermediate EOD frequencies, and during gonadal recrudescence, a rise in gonadal steroids induce opposite changes in the EOD of males and females.

EOD frequency can be shifted in the female direction by estrogen treatment in two gymnotiforms, *A. leptorhynchus* (Meyer et al. 1987; Schaefer and Zakon 1996) and *S. macrurus* (Dunlap et al. 1997). In *Eigenmannia virescens* and *A. albifrons*, estrogen appears to masculinize the EOD frequency (K. Dunlap, unpublished observations), and more studies are necessary to determine which hormones (or other cues) induce the female typical EOD.

Hypothesis for the evolution of sexual dimorphism in electric signals and its androgen regulation in Gymnotiformes

Complementary hormonal and behavioral studies have now been conducted on four wave-type gymnotiform species. The recent publication of a phylogeny of the Order based on 12S and 16S mitochondrial ribosomal RNA (Alves-Gomes et al. 1995) allows us to place species differences in a phylogenetic context and propose a hypothesis for the sequence of events in the evolution of sexual dimorphism in the electrocommunication system and its androgen regulation (Fig. 5).

In the two more ancestral species, *S. macrurus* and *E. virescens,* females discharge at higher frequencies than males. This pattern was retained in *A. albifrons*, but the direction of dimorphism was apparently reversed in *A. leptorhynchus*. Likewise, 11KT decreases EOD frequency in *Sternopygus*, *Eigenmannia* and *A. albifrons*, but increases EOD frequency in *A. leptorhynchus*. Thus, it appears that evolutionary changes in direction of sexual dimorphism have been accompanied, and perhaps caused by parallel evolutionary changes in the response to androgens.

Of the four species which have been examined, *Eigenmannia* is the most primitve species to display chirping, and in this species it is sexually dimorphic, with males chirping much more than females (Hopkins 1974). Chirping is not exhibited by either sex in the most ancestral species, *S. macrurus* (Keller et al. 1991.) *A. leptorhynchus* shows a dimorphism in chirping similar to *Eigenmannia*, but *A. albifrons* is apparently monomorphic in chirping (Fig. 1). Because chirping is dimorphic in the two other species which exhibit chirping, we interpret the absense of dimorphism in chirping in *A. albifrons* as an evolutionary loss of dimorphism. No studies have examined the androgen regulation of chirping in *Eigenmannia*, so it is difficult to polarize evolutionary changes in androgen sensitivity of chirping. Nevertheless, the absence of dimorphism in chirping in *A. albifrons* coincides with an absence of sensitivity to androgen, and it is reasonable to hypothesize that the apparent loss (or reduction) of dimorphism in chirping was caused by a loss (or reduction) of androgen sensitivity.

Apteronotidae is one of the most speciose and morphologically diverse families in the Order Gymnotiformes. Yet examination of the mitochondrial rRNA shows that species within the family are very closely related, with an average sequence divergence of only 3.67% (Alves-Gomes et al. 1995). Given this small divergence and the large differences in sexual dimorphism we report in just two species, it is likely that further comparative studies within Apteronotidae will reveal a remarkably rapid evolutionary diversification in the direction, magnitude and hormonal control of sexual dimorphism.

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