The Journal of Experimental Biology 216, 2421-2433

doi:10.1242/jeb.082933

REVIEW

Evolution and hormonal regulation of sex differences in the electrocommunication behavior of ghost knifefishes (Apteronotidae)

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Summary

The ghost knifefishes (family Apteronotidae) are one of the most successful and diverse families of electric fish. Like other weakly electric fish, apteronotids produce electric organ discharges (EODs) that function in electrolocation and communication. This review highlights the diversity in the structure, function and sexual dimorphism of electrocommunication signals within and across apteronotid species. EOD frequency (EODf) and waveform vary as a function of species, sex and/or social rank. Sex differences in EODf are evolutionarily labile; apteronotid species express every pattern of sexual dimorphism in EODf (males>females; males<females; males=females). The direction and magnitude of sex differences in EODf are correlated across species and populations with the responsiveness of EODf to androgens and/or estrogens, which suggests that sex differences evolve through gains and/or losses of hormone sensitivity. During social interactions, apteronotids also modulate their EODs to produce motivational signals known as chirps. Chirp structure differs markedly across species, and many species produce two or more discrete chirp types with potentially different functions. The structure of chirps is sexually dimorphic in all apteronotid species, and chirping is influenced by gonadal steroids and by neuromodulators. Encoding of chirps by the electrosensory system depends on the social context created by the interactions of the EODs of signalers and receivers. Electrosensory systems may thus influence the evolution of signal structure and function, and neuromodulators may coordinately shape the production and reception of electrocommunication signals depending on social context.

Key words: sexual dimorphism, communication, androgens, estrogens, neuromodulators, electric fish.

Received 12 November 2012; Accepted 31 March 2013

Introduction

The electric organ discharges (EODs) of weakly electric fish allow them to navigate and forage through dark and turbid waters and provide them with a private communication channel (Bullock, 1973; Lissmann, 1963). Each electric fish species generates either pulse-type EODs, with short duty cycles and often variable timing, or wave-type EODs with relatively constant frequencies and duty cycles of ~50% (Hopkins, 1974). Specialized electroreceptors in the skin detect perturbations in the temporally varying electric field of the EOD and allow the fish to perceive nearby objects or organisms (Bennett, 1971b). Electroreceptors can also respond to the EODs of other fish.

Several EOD parameters function as communication signals. EOD waveform, the temporal pattern with which the electric field changes during each discharge, varies across both South American gymnotiform and African mormyriform species. It also varies based on sex, reproductive status or social rank, particularly in species that have pulse-type EODs. In many species, EOD amplitude depends on the size and geometry of the electric organ, varies on multiple time scales, and responds to the physical and social environment (Hopkins, 1999; Stoddard, 2006). EOD amplitude can thus signal sex, body size and/or reproductive condition. In species that produce wave-type EODs, EOD frequency (EODf), or the rate at which the electric organ fires, also varies across species. In some species, EODf is sexually dimorphic and varies within sexes as a function of dominance. In most species with wave-type EODs, the EOD is emitted continuously, and EOD amplitude, waveform and frequency can thus serve as an information-rich badge that advertises the identity of the signaler.

Electric fish also transiently modulate the timing (in pulse-type EOD species), frequency (in wave-type EOD species) and/or amplitude of the EOD during social interactions. These EOD modulations (called chirps, rises, pings, yodels, interruptions, accelerations, decelerations, etc.) can function as motivational signals. This review highlights the evolution, hormonal and neural control, and function of EODs and chirps in one of the most successful radiations of Neotropical electric fishes, the ghost knifefishes (family Apteronotidae).

Electrical signaling in ghost knifefishes

The ghost knifefishes are the most speciose family of the South American electric fishes. The Apteronotidae includes more than 60 species (roughly a third of all gymnotiform species), and several new species are described each year (Crampton and Albert, 2005; Crampton and Albert, 2006). The ghost knifefishes have numerous adaptations that distinguish them from other electric fishes, including electric organs derived from nervous, rather than muscle, tissue and the exclusive use of electrical synapses in the neural circuit that controls EODf (see 'Neural control of EODf' below). These adaptations allow apteronotids to generate EODs at higher frequencies than those of other electric fishes. EOD waveform and frequency vary across apteronotid species (Table 1) (Crampton and

Table 1. Species differences in EOD and chirp parameters

Species	EODf range (Hz)	EOD waveform	Multiple chirp types
Orthosternarchus tamandua	ND	MPHN[1]	ND
Sternarchorhamphus muelleri	ND	MPHN[1]	ND
Platyurosternarchus macrostomus	ND	BPS-BPI[1]	ND
Sternarchorhynchus roseni	1181–1411 (8)[2]	TP[1,2]	FM/AM?[2]
Sternarchorhynchus curvirostris	815–1108 (7)[2]	TP[2,3]	FM/AM?[2]
Parapteronotus hasemani	773–982 (14)[2]	BPI[1,2]	Duration?[2]
Apteronotus leptorhynchus	697–927 (20)[4]	BPS-BPI[2]	FM/AM[4–7]
Apteronotus albifrons	865–1166 (42)[4]	BPS-BPI[1,2]	FM/AM[4,8]
Apteronotus magdalensis	509–949 (17)[9]	BPI[9]	ND
Magosternarchus raptor	ND	TP[1]	ND
Sternarchella schotti	ND	BPS-BPI[1,3]	ND
Sternarchella terminalis	1239–1325 (5)[2]	TP[2]	Chirp bursts *vs* singlets[2]
Sternarchella sp. 1	ND	TP[3]	ND
Apteronotus bonapartii	943–1679 (59)[2,10]	BPI[1,2]	Single- *vs* multi-peaked[2,10]
Apteronotus n. sp. B	1034 (1)[2]	TP[2]	Only one type?[2]
Compsaraia compsa	ND	BPI[1]	ND
Porotergus gymnotus	ND	BPS–BPI[1]	ND
Porotergus gimbelii	1149–1446 (9)[2]	BPS–BPI[2]	Only one type[2]
Sternarchogiton nattereri	910–1335 (9)[2]	BPI–TP[1,2]	FM/AM?[2]
Sternarchogiton porcinum	944 (1)[2]	BPI[2]	Only one type?[2]
Adontosternarchus sachii	ND	BPI[1]	ND
Adontosternarchus balaenops	803–1020 (10)[2]	BPI[2]	FM/AM[2]
Adontosternarchus devenanzii	978–1245 (21)[11]	BPI[2]	Single- *vs* multi-peaked[11]

ND, not determined.

Electric organ discharge frequency (EODf) range values were temperature corrected (Q_{10}=1.64) to that expected at 26°C (numbers in parentheses indicate sample size).

EOD waveform abbreviations: MPHN, monophasic, head negative; BPS, biphasic, sinusoidal (power of second harmonic – fundamental < –10 dB); BPI, biphasic with prominent inflection (power of second harmonic – fundamental between 0 and –10 dB); TP, triphasic (power of second harmonic > power of fundamental).

Multiple chirp type parameters: FM, frequency modulation; AM, amplitude modulation. A question mark indicates small sample size and/or ambiguous distinction between chirp types.

[1]Crampton and Albert, 2006; [2]Turner et al., 2007; [3]Kramer et al., 1981; [4]Kolodziejski et al., 2005; [5]Engler and Zupanc, 2001; [6]Hagedorn and Heiligenberg, 1985; [7]Zupanc and Maler, 1993; [8]Dunlap and Larkins-Ford, 2003a; [9]Maldonado-Ocampo et al., 2011; [10]Ho et al., 2010; [11]Zhou et al., 2006.

Albert, 2006; Kramer et al., 1981; Turner et al., 2007), which may allow EODs to be used as species-identification signals. All apteronotids modulate their EODs during social interactions to produce chirps and rises, and the structure of chirps also differs across species (Turner et al., 2007). EOD and chirp parameters also vary considerably within species as a function of sex, condition or social rank. Furthermore, patterns of within-species variation differ across species in interesting ways, including gains, losses and even reversals in the direction of sexual dimorphism. The species diversity in both the signals themselves and the sexual dimorphism of the signals makes the electrocommunication behavior of ghost knifefishes a model well-suited for addressing questions on sexual selection and the evolution of communication. Furthermore, the responsiveness of EODs and chirping to hormones and the relative simplicity of the neural circuits that regulate these behaviors makes them an outstanding model for understanding the neural and hormonal control of sexually dimorphic behavior (reviewed in Zakon, 1993; Zakon and Smith, 2009).

Sex differences in EOD frequency

Sexual dimorphism of EODf is evolutionarily labile in apteronotid species. In non-apteronotid species with wave-type EODs (e.g. *Eigenmannia* sp. and *Sternopygus* sp.), males have lower EODfs than females (Dunlap and Zakon, 1998; Hopkins, 1972). Apteronotid species vary in both the magnitude and direction of sexual dimorphism in EODf. The brown ghost knifefish (*Apteronotus leptorhynchus*) has a robust sex difference in EODf in the opposite direction to that of other knifefishes; males have higher EODf than females (Dunlap et al., 1998; Hagedorn and Heiligenberg, 1985; Meyer et al., 1987). In contrast, male black ghost knifefish (*Apteronotus albifrons*) have lower EODfs than females (Dunlap et al., 1998). Furthermore, the strength of the sex difference in EODf varies across *A. albifrons* populations. In *A. albifrons* populations from the Orinoco river system, male EODfs are ~200 Hz lower than female EODfs, and EODf overlaps little between the sexes. In contrast, sex differences in EODf in Amazonian populations of *A. albifrons* are modest (~50 Hz), with more extensive overlap between the sexes (Ho et al., 2013). In the majority of other apteronotid species that have been studied, EODf does not differ between the sexes (Table 2) (Ho et al., 2010; Maldonado-Ocampo et al., 2011; Petzold and Smith, 2012b; Zhou and Smith, 2006). EODf thus shows every possible pattern of sexual dimorphism across apteronotid species, and within species, populations also vary in sexual dimorphism. This suggests that sex differences in EODf have evolved rapidly in apteronotids.

Apteronotid species vary markedly in morphological sexual dimorphism. Some species are highly sexually dimorphic, with males being larger than females and having adaptations for fighting. Extreme cases of morphological sexual dimorphism include *Parapteronotus hasemani*, in which males with extremely elongated jaws were previously misclassified as a separate species (*Apteronotus anas*) (Cox Fernandes et al., 2002), and *Sternarchogiton nattereri*, in which males with a ball of external teeth on the upper jaw were previously misclassified in a separate

Table 2. Species differences in sexual dimorphism and hormonal control of EODs and chirping

Species	Sex difference in EODf	Steroid effect on EODf	Sex difference in chirp rate	Steroid effect on chirp rate	Sex difference in chirp structure	Steroid effect on chirp structure
Parapteronotus hasemani	M=F[6]	AND, FLT–0[6]	M≥F[1,6]	FLT–0; AND–↑?[2,6]	Duration: M>F[6]	FLT: ↓duration[6]
Apteronotus leptorhynchus	M>>F[7,8]	AND–↑[9]; FLT–↓[10] EST–↓[9]; TMX–↑[10]	M>>F[11,12]	AND–↑[13,14]	AM,FM: M>F[3,7,15,16]	AND: ↑AM,FM[13,14]
Apteronotus albifrons (Orinoco)	M<<F[8,17]	AND–↓[8,17]	M=F[8,17]	AND–0[8,17]	Duration: M>F[16,18]	ND
Apteronotus albifrons (Amazon)	M≤F[4,17]	AND–↓[4,17]	M=F[17]	AND–0[17]	ND	ND
Apteronotus magdalensis	M=F[19]	ND	ND	ND	ND	ND
Apteronotus bonapartii	M=F[20]	AND–0?[2,21]	M=F[20]	ND	Multi-peaked chirps: M>F[20]	ND
Sternarchogiton nattereri	M(t)>M(n)=F[5,22]	ND	M=F[23]	ND	AM,FM: M>F[23]	ND
Adontosternarchus devenanzii	M=F[24]	AND–0?[2,21]	M=F[24]	ND	Multi-peaked chirps: M>F[24]	ND

The steroid effect is indicated as: 0, no effect; ↑, increase; ↓, decrease. M, male; F, female; AND, androgen; EST, estrogen; FLT, flutamide (androgen receptor blocker); TMX, tamoxifen; ND, not determined. A question mark indicates small sample size and/or marginally significant result.

[1]Marginally significant result (*t*=test, *P*=0.08); [2]small sample size (*N*≤3); [3]males produce high-frequency chirps that are rarely produced by females – male low-frequency chirps have greater FM; [4]sex difference and androgen effect are significantly smaller in Amazon populations than in Orinoco populations; [5]males with teeth have higher EODf than non-toothed males or females; [6]Petzold and Smith, 2012a; [7]Hagedorn and Heiligenberg, 1985; [8]Dunlap et al., 1998; [9]Schaefer and Zakon, 1996; [10]Fernandez and Smith, 2012; [11]Dye, 1987; [12]Zupanc and Maler, 1993; [13]Dulka and Maler, 1994; [14]Dulka et al., 1995; [15]Bastian et al., 2001; [16]Kolodziejski et al., 2005; [17]Ho et al., 2012; [18]Dunlap and Larkins-Ford, 2003a; [19]Maldonado-Ocampo et al., 2011; [20]Ho et al., 2010; [21]G.T.S. and M. Zhou, unpublished observations; [22]Cox Fernandes et al., 2010; [23]Formby et al., 2009; [24]Zhou and Smith, 2006.

genus (*Oedemognathus exodon*) (Cox Fernandes et al., 2009). Other apteronotid species (e.g. *Adontosternarchus devenanzii*) are sexually monomorphic in body size and shape (Zhou and Smith, 2006).

Morphological sex differences are poorly correlated with sex differences in EODf across species. *Apteronotus leptorhynchus*, which has the most pronounced sex difference in EODf, is also highly sexually dimorphic in body size and head shape (Hagedorn and Heiligenberg, 1985). However, some populations of *A. albifrons* also have large sex differences in EODf, but are only moderately sexually dimorphic in body size and shape (Dunlap et al., 1998). In *S. nattereri*, EODf differs between male morphs. Males with external teeth have higher EODf than females or reproductively mature males without teeth (Cox Fernandes et al., 2010). However, in *P. hasemani*, which has extreme within- and between-sex variation in head morphology, EODf is sexually monomorphic and does not differ between long-jawed and short-jawed males (Petzold and Smith, 2012b). Thus, sexual selection for body size dimorphism and weaponry is not linked consistently to selection for sex differences in EODf.

Hormonal regulation of EODf

Sex differences in EODf are influenced by gonadal hormones. In the few species where hormonal regulation of the EOD has been studied, activational effects of steroids on EODf parallel sex differences (Table 2). In species lacking sex differences in EODf (*P. hasemani*, *Apteronotus bonapartii* and *A. devenanzii*), treatment with 11-ketotestosterone (11KT, the primary non-aromatizable androgen in fishes) and/or blocking androgen receptors with flutamide does not affect EODf (Petzold and Smith, 2012a) (G.T.S., J. Petzold and M. Zhou, unpublished observations). In *A. leptorhynchus*, EODf is higher in males than in females, 11KT increases EODf, and flutamide lowers EODs (Fernandez and Smith, 2012; Schaefer and Zakon, 1996). In *A. albifrons*, EODf is lower in males than in females, and 11KT lowers EODf (Dunlap et al., 1998). Furthermore, the relationship between androgen effects and sex differences in EODf is consistent across populations within species. 11KT robustly lowers EODf in populations of *A. albifrons* that are highly sexually dimorphic, whereas EODf is significantly less responsive to 11KT in populations of *A. albifrons* with smaller sex differences in EODf (Ho et al., 2013). These findings suggest that species diversity in the sexual dimorphism of EODf may evolve in part through the gain or loss of androgen sensitivity, or through the reversal of androgenic effects.

In some species, androgens may also regulate within-sex variation in EODf. EODf was positively correlated with plasma 11KT levels in male *A. leptorhynchus* that were individually housed and then exposed to a series of short (10 min) dyadic interactions (Dunlap, 2002). In contrast, excreted 11KT concentrations were not correlated with EODf in male *A. leptorhynchus* housed in small social groups, although there was a trend for changes in 11KT induced by reduced conductivity to correlate positively with changes in EODf (Cuddy et al., 2012). In *S. nattereri*, reproductively mature males with external teeth have larger testes, higher levels of 11KT and higher EODf than reproductively mature males without teeth (Cox Fernandes et al., 2010). In contrast, 11KT levels are not correlated with EODf, size or jaw length in male *P. hasemani* and *A. bonapartii*, which have also have high within-sex morphological variation (Ho et al., 2010; Petzold and Smith, 2012b). These findings suggest that androgens might provide a mechanism that links within-sex morphological variation with EODf in some, but not all, apteronotid species.

Somewhat paradoxically, testosterone feminizes EODf in *A. leptorhynchus* (Dulka and Maler, 1994). The feminizing effect of testosterone on EODf is likely mediated by aromatization and estrogen receptors. Blocking androgen receptors also feminizes EODf, and the feminizing effects of testosterone on EODf are blocked by aromatase inhibitors (Fernandez and Smith, 2012; Zucker, 1998). Testosterone has no effect on EODf in *A. albifrons*, despite the fact that other androgens masculinize EODf (Dunlap et al., 1998). The lack of an effect of testosterone on EODf in *A. albifrons* could result from either the differential activation of androgen receptors by testosterone *versus* 11KT or counterbalancing androgenic and estrogenic effects. Although male apteronotids have plasma 11KT levels that are severalfold greater than those of females, females' testosterone levels are typically as high as those in males. This suggests that testosterone may serve as a hormone of reproductive condition in both sexes rather than as

a sex-specific hormone (Cox Fernandes et al., 2010; Dunlap et al., 1998; Ho et al., 2010; Petzold and Smith, 2012b).

In *A. leptorhynchus*, sex differences in EODf are also regulated by estrogens. Estradiol feminizes (lowers) EODf (Schaefer and Zakon, 1996). Furthermore, inhibiting estrogen synthesis with CGS16949A or blocking estrogen receptors with tamoxifen defeminizes (elevates) EODf (Fernandez and Smith, 2012; Zucker, 1998). Thus, sex differences in EODf are regulated by an androgenic–estrogenic push–pull system. The role of estrogens in regulating EODf in other apteronotid species, however, is not known.

Neural control of EODf

The electric organs of adult ghost knifefish, unlike those of other electric fish, consist of highly modified peripheral nerves. Spinal electromotor neurons (EMNs), whose axons form the electric organ, differ from other motor neurons in several ways. Their axons emerge from the ventral roots of the caudal spinal cord with relatively conventional, excitable nodes of Ranvier. Within the electric organ, however, the axons dilate to a diameter of about 100 μm (i.e. greater in diameter than EMN cell bodies), and the axons have huge (~50 μm long), passive nodes (Bennett, 1971a). Narrower axons and smaller, active nodes that regenerate action potentials are present at 'hairpin' turns of the axons within the electric organ. EMN axons are also unusual in being targetless and ending blindly. EMN action potentials generate the EOD as capacitative currents across the large, passive nodes. The capacitative currents of the apteronotid EOD create an added advantage by filtering out low frequencies that are conspicuous to predators with ampullary electroreceptors (e.g. catfish) (Bennett, 1971a; Stoddard, 1999).

The neural circuit that controls EODf is relatively simple (Fig. 1) (reviewed in Smith, 1999). EODf is regulated by three neuron types: pacemaker and relay neurons in the medullary pacemaker nucleus (PMN) and EMNs in the spinal cord. The endogenous firing rate of these neurons corresponds directly with EODf, and changes in their firing rates are translated directly into changes in EODf (Bennett, 1971a; Elekes and Szabo, 1985; Szabo and Enger, 1964). A relatively simple suite of ionic currents, including transient Na^+ currents with ultra-rapid kinetics, persistent Na^+ currents, and K^+ currents carried by Kv1-like channels, allow pacemaker and electromotor neurons to fire spontaneously at such high rates (Dye, 1991; Smith, 2006; Smith and Zakon, 2000). In *A. leptorhynchus*, androgenic and estrogenic effects on the firing rates of electromotor and pacemaker neurons underlie the sex difference in EODf. Adult treatment with 11KT increases EODf by increasing the spontaneous firing rates of the PMN and EMNs; and estradiol decreases EODf by decreasing their firing rates (Schaefer and Zakon, 1996). The electromotor system of apteronotids thus provides a strong model system for investigating the cellular mechanisms by which steroid hormones regulate sex differences in behavior.

The electromotor system of apteronotids is built for speed. Pacemaker, relay and electromotor neurons are extensively electrically coupled with no chemical synapses between them (Bennett et al., 1978; Elekes and Szabo, 1985). The absence of chemical synapses in the electromotor system reduces synaptic delays and enhances firing synchrony. These adaptations allow ghost knifefish to fire their electric organs precisely at rates approaching 2000 Hz (Kramer et al., 1981; Moortgat et al., 1998). Indeed, neurons in the apteronotid electromotor system fire at the highest sustained rates of any neurons in the animal kingdom and can thus serve as a model for understanding the energetics and ionic mechanisms of high-frequency neuronal activity. The fast EODs of ghost knifefish potentially increase sampling rates for electrolocation and have opened up new bandwidths for their electrocommunication signals.

Signal function of EODs

EOD waveform and frequency as species identifiers

EODf varies over roughly a fourfold range (~500–2000 Hz) across apteronotid species. Particularly at locales with relatively few electric fish species, EODf may signal species identity (Hopkins, 1974; Hopkins and Heiligenberg, 1978). The within-species variation in EODf, however, can be quite large. EODf in *A. bonapartii* spans 600 Hz (1000–1600 Hz), and EODf commonly spans 200–300 Hz in other species (Table 1) (Kramer et al., 1981; Turner et al., 2007). Large within-species variation in EODf and the species richness of apteronotids at many sites result in extensive overlap of EODf across sympatric species, which limits the utility of EODf alone as a species-identification signal.

Unlike other wave-type gymnotiforms, which produce monophasic EODs, apteronotids produce EODs with complex waveforms that vary across species (Table 1) (Crampton and Albert, 2006; Kramer et al., 1981; Turner et al., 2007). EOD waveform complexity in apteronotids results from a different mechanism than waveform complexity in pulse-type electric fish, which have muscle-derived electric organs. The apteronotid EOD is generated by action potentials synchronously propagating along EMN axons in the electric organ. The trajectory of those axons is correlated with EOD waveform (Bennett, 1971a) (H. Kincaid and C. Cox Fernandes, personal communication). In species with biphasic EODs (e.g. *A. albifrons* and *Platyurosternarchus*), EMN axons initially project anteriorly in the electric organ and then loop back in a 'hairpin turn' to project caudally. The rostral propagation of action potentials through the proximal portion of the axons generates the head-positive phase of the EOD, and the caudal propagation of action potential through the distal portion of the axons generates the head-negative phase. In species with monophasic head-negative EODs (*Sternarchorhamphus* and *Orthosternarchus*), the EMN axons lack the rostrally running axon segments and project only caudally within the electric organ. In these species, action potentials travel only caudally within the axons, generating the monophasic head-negative EOD.

Because EOD waveforms vary across apteronotid species, but are relatively stereotyped within species, waveform could function as a signal of species identity. A discriminant function analysis based on EOD parameters in 12 apteronotid species was able to predict species identity in most cases (Turner et al., 2007). EOD waveform and frequency thus contain information that might allow fish to discriminate conspecific from heterospecific individuals. Few studies, however, have measured the ability of apteronotids to discriminate between EOD waveforms. Male *A. leptorhynchus* chirped more at playbacks of pure sine waves than at playbacks of conspecific EODs of the same frequency and amplitude (Dunlap and Larkins-Ford, 2003b). Thus, fish discriminated between different EOD waveforms, but signals with conspecific waveforms were less effective at evoking chirps than pure sine waves. Male *A. leptorhynchus* approached and chirped more towards playback stimuli with conspecific *versus* heterospecific EOD frequencies, but did not behave differently towards playbacks with conspecific *versus* heterospecific EOD waveforms (Fugère and Krahe, 2010). These results suggest that at least for chirping and approach, male *A. leptorhynchus* do not prefer conspecific EOD waveforms.

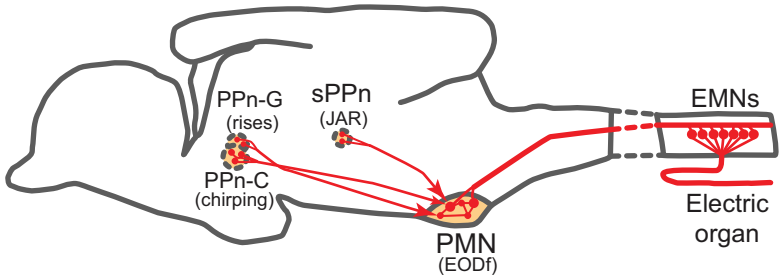

Fig. 1. Sagittal schematic diagram of the apteronotid electromotor system. Electric organ discharge frequency (EODf) is regulated by the pacemaker nucleus (PMN) in the hindbrain. Pacemaker and relay cells in the PMN are electrotonically coupled. Relay cell axons project down the spinal cord and form electrical synapses on electromotor neurons (EMNs), whose specialized and targetless axons fasciculate to form the electric organ. The PMN receives descending glutamatergic input from three sources: projection neurons in the 'chirp' subdivision of the thalamic prepacemaker nucleus (PPn-C) synapse on relay cells and initiate chirps. Neurons in a more rostromedial subdivision of the prepacemaker nucleus (PPn-G) project to pacemaker cells and initiate rises. The midbrain sublemniscal prepacemaker nucleus (sPPn) also projects to the PMN and regulates the jamming avoidance response (JAR).

Further studies are needed to determine whether *A. leptorhynchus* can discriminate between EOD waveforms in other behavioral contexts (e.g. female mate choice) or whether other apteronotid species respond differentially to EOD waveforms.

EODf as a signal of sex

EODf is sexually dimorphic in opposite directions in two well-studied apteronotid species. EODf is greater in males than in females in *A. leptorhynchus*, and lower in males than in females in *A. albifrons* (Dunlap et al., 1998). Sex differences in EODf may have evolved through sexual selection, and EODf may serve as a reliable signal of sex in these species. Direct evidence of EODf functioning as a sexual signal in apteronotids, however, is relatively sparse.

The strongest evidence that EODf signals sex is that fish chirp differently to male *versus* female EOD playbacks. In *A. leptorhynchus* and *A. albifrons*, fish consistently produce more low-frequency (type 2) chirps in response to EODfs similar to the fish's own (i.e. same-sex EODfs) than in response to EODfs that differ more from their own (e.g. opposite-sex EODfs) (Bastian et al., 2001; Bohorquez and Smith, 2011; Cuddy et al., 2012; Dye and Heiligenberg, 1987; Engler and Zupanc, 2001; Hupé and Lewis, 2008; Kolodziejski et al., 2007; Triefenbach and Zakon, 2003; Zupanc et al., 2006). Low-frequency chirps have been hypothesized to function as agonistic signals (see 'Function of chirps' below). The preferential production of low-frequency chirps in response to EODfs close to a fish's own is consistent with the fish using EODf as a cue to direct agonistic chirps towards individuals of the same sex.

Male *A. leptorhynchus* produce high-frequency chirps, which are hypothesized to function as courtship signals (Hagedorn and Heiligenberg, 1985), more in response to EODfs far from their own than to EODfs similar to their own (Bastian et al., 2001; Kolodziejski et al., 2007; Triefenbach and Zakon, 2003). By producing high-frequency chirps to EODfs 100–200 Hz below their own EODf, males may be using EODf to direct these chirps preferentially to females. A potentially confounding point, though, is that males also produce high-frequency chirps in response to EODfs 100–200 Hz above their own (Cuddy et al., 2012; Engler et al., 2000). This suggests either that fish are unable to discriminate between EODfs far above *versus* below their own EOD or that high-frequency chirps might not function exclusively as intersexual signals (see 'Function of chirps' below).

EODf does not differentially influence chirping in species that lack sex differences in EODf. *Apteronotus bonapartii* and *A. devenanzii*, whose EODfs are sexually monomorphic, produce similar numbers of chirps in response to playbacks across the species-typical range of EODfs (Ho et al., 2010; Zhou and Smith, 2006). Thus, in apteronotid species where EODf contains information about sex (*A. leptorhynchus* and *A. albifrons*), fish respond differently to playbacks of male-typical *versus* female-typical EODfs, whereas in species where EODf does not contain information about sex (*A. bonapartii* and *A. devenanzii*), fish do not chirp differently in response to different EODfs.

EODf as an intrasexual signal of social rank

EODf may also act as a signal of social rank in *A. leptorhynchus*. EODf is often correlated positively with body size in males (Cuddy et al., 2012; Dunlap, 2002; Dunlap and Oliveri, 2002; Triefenbach and Zakon, 2003) (but see Triefenbach and Zakon, 2008). Some studies have also found that males and/or females with higher EODf are dominant in competitions over shelter tubes or access to mates (Dunlap and Oliveri, 2002; Hagedorn and Heiligenberg, 1985), whereas other studies did not find higher EODf in males or females that win aggressive dyadic contests (Bohorquez and Smith, 2011; Triefenbach and Zakon, 2008). Male and female *A. leptorhynchus* were more likely to bite an electrode playing simulated EODs with frequencies 15 Hz below their own EODf than those playing 15 Hz above their own EODf, which suggests that lower EODfs may signal subordinance (Tallarovic and Zakon, 2005). Differences between studies in the relationship between EODf and dominance could result from differences in experimental design, reproductive condition, and/or social environment. For example, social experience can increase EODf independently of social rank (Bohorquez and Smith, 2011).

Relatively few studies have examined the relationship between EODf and body size, dominance or aggression in other apteronotid species. *Sternarchogiton nattereri* males that have external teeth, which are used in male–male aggression, have higher EODf than males without teeth (Cox Fernandes et al., 2010). However, in male *P. hasemani*, EODf is not significantly correlated with body size, and males with long jaws do not differ in EODf from males with short jaws (Petzold and Smith, 2012b). In reproductively regressed *Sternarchorynchus* sp., EODf was positively correlated with body size and dominance in dyadic trials; and fish were more likely to approach and attack EOD playbacks of frequencies lower than their own (Fugère et al., 2011). These studies suggest that in some, but not all, apteronotid species, EODf may serve as a signal of competitive ability and/or dominance.

Sex and species differences in chirps and rises

Every apteronotid species that has been studied produces transient modulations of their EOD during social interactions. EOD modulations fall into two broad categories. Chirps are rapid, relatively stereotyped, and short-duration increases of tens to hundreds of Hz in EODf. Chirps, particularly those with greater frequency modulation, are often accompanied by decreases in EOD amplitude. Rises (also called gradual frequency rises) are slower, smaller (few to tens of Hz), longer (hundreds of ms to s), and more variable increases in EODf.

Rises

The properties of rises (i.e. duration, frequency modulation and complexity) are highly variable within species, but show little predictable variation across species. Consequently, rises convey little information about species identity (Turner et al., 2007). The function of rises is unclear, in part because their variability has made them difficult to categorize and compare across studies. Unlike chirps, rises are often sexually monomorphic (Ho et al., 2010; Kolodziejski et al., 2005; Zhou and Smith, 2006). Also unlike chirps, EOD playbacks to fish in chirp chambers inhibit rather than stimulate the production of rises in *A. leptorhynchus*, *A. albifrons* and *A. bonapartii* (Ho et al., 2010; Kolodziejski et al., 2007).

In *A. albifrons*, rises are produced more often by submissive than by dominant fish in live, dyadic interactions, which suggests that they may function as appeasement signals in this species (Serrano-Fernández, 2003). In contrast, rises in *A. leptorhynchus* have been proposed as signals of either dominance or subordinance. These fish produce more rises in response to EODfs lower than their own in live interactions or interactions *via* wires connecting the fishes' tanks (Tallarovic and Zakon, 2002; Tallarovic and Zakon, 2005). If higher EODf is an indicator of dominance (see above), this suggests that dominant fish produce more rises than subordinates. Rise production coincides with attacks in live interactions, although both the aggressor and the fish being attacked may produce them (Hupé and Lewis, 2008; Triefenbach and Zakon, 2008). Losing fish produce more rises than winning fish shortly after a conflict, which suggests that some types of rises might also function as signals of subordinance. One possibility is that rises are a general signal of social stress that may be expressed by either individual during critical phases of conflict. Yodels, a type of rise in which EODf rises abruptly by tens of Hz and then slowly and exponentially decays back to baseline, are commonly produced when playback stimuli are terminated and have also been observed in female–female competition (Dye, 1987; Zakon et al., 2002). Dye hypothesized that yodels function as 'victory cries' emitted by a fish when an intruder (i.e. the playback) was vanquished and retreated (Dye, 1987).

Chirps

Across-species variation in chirp structure

Nearly every parameter of chirp structure varies within and across apteronotid species (Table 1, Fig. 2) (Turner et al., 2007). The average frequency modulation (FM, increase in EODf) of chirps ranges from less than 70 Hz (*A. leptorhynchus* and *Sternarchorhynchus roseni*) to more than 400 Hz (*P. hasemani*). Average chirp duration varies over more than an order of magnitude, from less than 30 ms (*A. leptorhynchus* and *Sternarchella terminalis*) to more than 500 ms (*P. hasemani*). Amplitude modulation (AM) of chirps varies from none to nearly 100% (i.e. the EOD is completely extinguished during most chirps of *P. hasemani* and many chirps in *Porotergus gimbelii*). Chirp AM is strongly correlated with FM, such that chirps that have little FM have little AM, whereas chirps with large increases in EODf have large decreases in EOD amplitude.

Chirps also vary in spectrotemporal complexity and/or patterning. Most apteronotid chirps have a simple structure: a rapid increase in EODf followed by a slower return to baseline EODf. Some species (e.g. *A. leptorhynchus* and *A. bonapartii*) produce chirps in which the EODf increase is followed by a pronounced EODf undershoot. Some chirps produced by *A. devenanzii*, *A. bonapartii* and *A. albifrons* have multiple frequency peaks (Dunlap and Larkins-Ford, 2003a; Turner et al., 2007; Zhou and Smith, 2006). In live interactions, *A. leptorhynchus* produces chirps in a non-random pattern that includes chirp bursts and interactive chirping (Hupé and Lewis, 2008; Zupanc et al., 2006). Bursty chirp production is particularly pronounced in *S. terminalis*, which produces intense chirp bursts riding on top of a 5–15 Hz plateau-like elevation of EODf (Turner et al., 2007).

The marked interspecific variation in chirp structure suggests that chirps are rapidly evolving and, like EOD waveform, could potentially act as a species-identifying signal. A discriminant function analysis with six chirp parameters (AM, FM, duration, undershoot FM, and positive and negative FM slopes) in 12 apteronotid species correctly identified the species of most individuals (Turner et al., 2007). Additional studies are needed to determine whether chirp parameters are actually used to discriminate between conspecific and heterospecific fish.

Within-species variation in chirp structure

Chirps vary substantially within species. In some species, chirp parameters vary discontinuously to form discrete 'types' of chirps (Table 1, Fig. 2). *Apteronotus leptorhynchus*, for example, produces at least two different chirp types (Bastian et al., 2001; Hagedorn and Heiligenberg, 1985; Zupanc and Maler, 1993). The most common chirps (called low-frequency chirps, small chirps or type 2 chirps) involve moderate increases in EODf (~20–150 Hz) and little AM. High-frequency chirps (also called big chirps or type 1 chirps) involve much greater increases in EODf (200–400 Hz), substantial decreases in EOD amplitude, and frequency undershoots of tens of Hz. High-frequency chirps have sometimes been subdivided into three types (types 1, 3 and 4) based on variation in duration and the frequency undershoot (Engler et al., 2000; Engler and Zupanc, 2001). Type 3 and 4 chirps are rare (<1% of chirps) (Engler and Zupanc, 2001; Kolodziejski et al., 2005), however, and it is unclear whether they have distinct functions or are one end of a continuum of high-frequency chirps. Two other chirp types (types 5 and 6) have been reported in interacting male *A. leptorhynchus* (Zupanc et al., 2006). These chirp types had large EODf increases and AM like type 1, 3 and 4 chirps, but were very long in duration and included periods with large, abrupt EODf decreases.

Apteronotus albifrons, like *A. leptorhynchus*, produces distinct high-frequency and low-frequency chirps (Fig. 2). The chirps of *A. albifrons*, however, are much longer in duration than those of *A. leptorhynchus*, and *A. albifrons* high-frequency chirps lack frequency undershoots (Dunlap and Larkins-Ford, 2003a; Kolodziejski et al., 2005). Several other species (e.g. *Adontosternarchus balaenops*) also produce chirps that may fall into discrete high- and low-frequency categories, although chirp types have not been well studied in these species (Turner et al., 2007). In other apteronotid species, distinct chirp types are based on parameters other than AM and FM. For example, *A. devenanzii*, *A. bonapartii* and *A. albifrons* produce simple chirps, with single

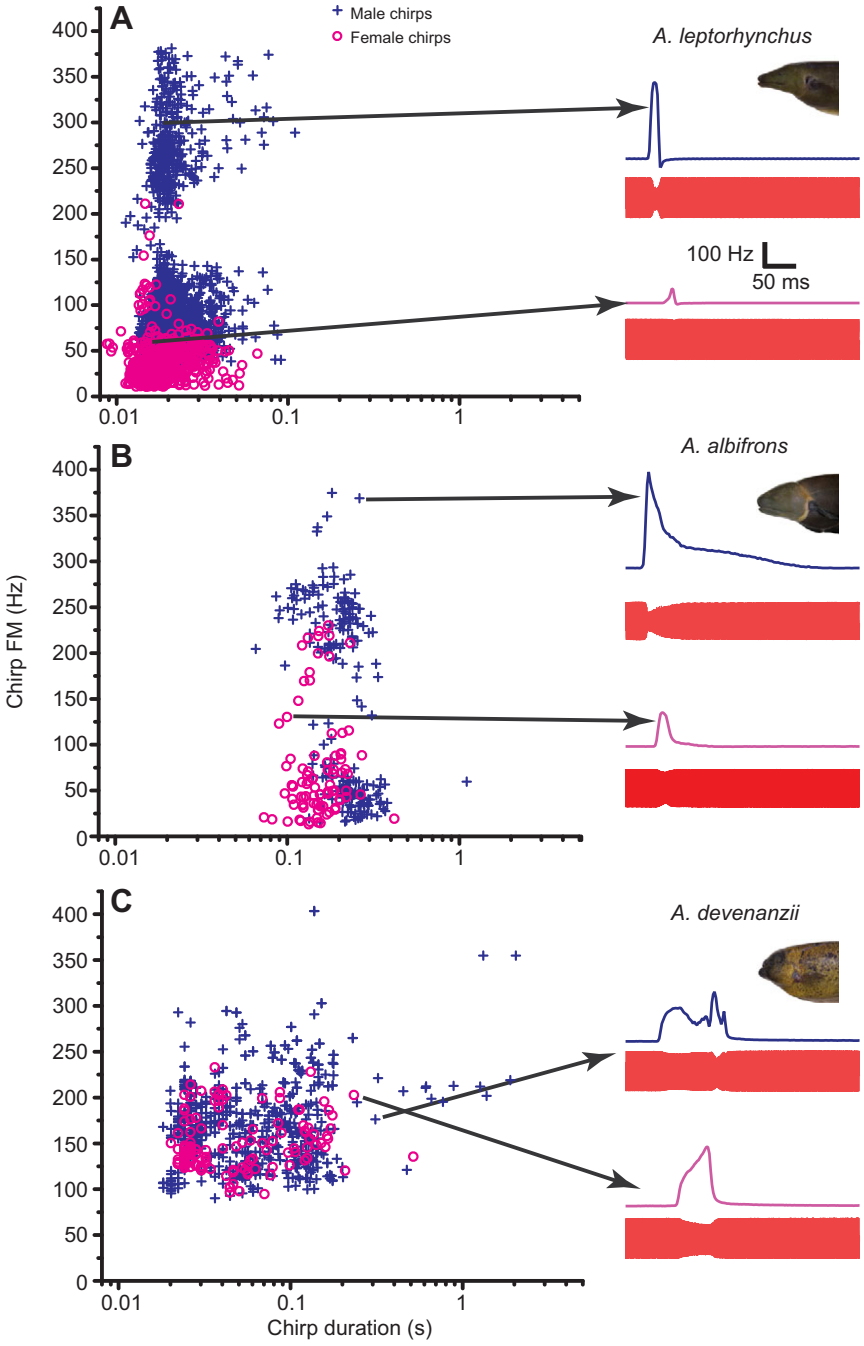

Fig. 2. Species and sex differences in chirp structure. Scatter plots show the frequency modulation (FM) and duration (log-scale) of chirps produced by males (blue crosses) and females (pink circles) in three apteronotid species. The axis scale is the same in all three graphs to allow comparisons across species. Examples of male and female chirps are shown on the right. The top (blue or pink) trace shows EOD frequency, and the bottom (red) trace shows head–tail voltage to illustrate the EOD amplitude envelope during the chirp. (A) *Apteronotus leptorhynchus* produces chirps with shorter durations than those of the other two species. High-frequency chirps are almost exclusively produced by males, and the FM of low-frequency chirps is also greater in males. Note the pronounced amplitude modulation (AM) in the envelope of the high-frequency chirp. (B) Chirps are nearly an order of magnitude longer in duration in *A. albifrons* compared with *A. leptorhynchus*. As in *A. leptorhynchus*, *A. albifrons* males produce more high-frequency chirps than females, and male chirps are also nearly twice as long as female chirps. High-frequency chirps have pronounced AM, but the AM lasts longer and has a more gradual offset. (C) Chirp duration in *A. devenanzii* is highly variable. Male and female chirps do not differ significantly in FM or duration, but males produce many more multi-peaked chirps. Note the complex AM of the multipeaked chirps that parallels the pattern of FM. Based on previously published data (Kolodziejski et al., 2005; Zhou and Smith, 2006).

frequency peaks like the chirps of *A. leptorhynchus*, and complex chirps that have multiple frequency peaks (Dunlap and Larkins-Ford, 2003a; Turner et al., 2007; Zhou and Smith, 2006). In other species (e.g. *P. gimbelii*), chirps vary continuously in FM, AM and/or duration and are difficult to categorize unambiguously into discrete types. Whether it is continuous or discrete, the within-species variation in chirp structure is substantial in most species and may allow chirps to convey information about sex, condition, motivation or rank.

Sex differences in chirping

Chirp rate is sexually dimorphic in *A. leptorhynchus*. In response to playbacks in a 'chirp chamber', males chirp several times more often than females (Bastian et al., 2001; Dye and Heiligenberg, 1987; Zupanc and Maler, 1993). Most studies of freely swimming *A. leptorhynchus* have also found higher rates of chirping in males than in females (Dunlap, 2002; Hupé and Lewis, 2008; Triefenbach and Zakon, 2003), although a recent study found that after 1 week of housing in small social groups, chirp rate during live dyadic trials did not differ significantly between the sexes, and females tended to chirp more than males (Bohorquez and Smith, 2011). This suggests that social experience and familiarity may interact with sex to influence chirp rate. Habituation to prolonged exposure to the EODs of social partners may in part mediate the effects of social experience on chirp rate. Chirp rate habituates strongly to repeated EOD playbacks, and this habituation is specific to the EOD frequency that is used to elicit chirping (Harvey-Girard et al., 2010). Chirp rate is sexually monomorphic in many other ghost knifefishes. In the five other apteronotid species that have been recorded in chirp chambers, chirp rate did not differ significantly

between males and females (Dunlap et al., 1998; Formby et al., 2009; Ho et al., 2010; Petzold and Smith, 2012b; Zhou and Smith, 2006).

Although EODf and chirp rates are sexually monomorphic in many apteronotid species, the structure of chirps differs between the sexes in every apteronotid species that has been studied (Table 2, Fig. 2). The nature of sex differences in chirp structure varies across species. In *A. leptorhynchus*, males produce high-frequency chirps, which are produced very rarely by females, and the low-frequency chirps of females have less FM than male low-frequency chirps (Fig. 2) (Bastian et al., 2001; Kolodziejski et al., 2005). Males also produce chirps with greater FM in *A. albifrons* and *S. nattereri* (Formby et al., 2009; Kolodziejski et al., 2005). In *A. albifrons* and *P. hasemani*, chirps are sexually dimorphic in duration; male chirps are longer than those of females (Dunlap and Larkins-Ford, 2003a; Kolodziejski et al., 2005; Petzold and Smith, 2012b). In *A. bonapartii* and *A. devenanzii*, the complexity of chirps differs between the sexes. Males produce more chirps with multiple frequency peaks, whereas females produce primarily single-peaked chirps (Ho et al., 2010; Zhou and Smith, 2006). The fact that sexual dimorphism of chirps is widespread, but that different parameters have been elaborated in males across different species, suggests that chirps have evolved rapidly. If chirps function as intrasexual agonistic signals or as courtship signals, sexual selection may have driven the rapid diversification of chirp structure.

Hormonal regulation of chirping

Sex differences in chirp rate and structure are regulated in part by activational effects of androgens. In *A. leptorhynchus* males interacting electrically but not physically with another fish, chirp rates were positively correlated with plasma levels of 11KT (Dunlap, 2002). In another study that used chirp chamber recordings, however, excreted 11KT levels were not significantly correlated overall with the production of either high-frequency or low-frequency chirps by male *A. leptorhynchus* (Cuddy et al., 2012). Treatment of female *A. leptorhynchus* with androgens partially masculinizes chirping (i.e. increases chirp rate, FM and AM), although androgenized females still chirp less than males (Dulka and Maler, 1994; Dulka et al., 1995). Combined, these results suggest that (1) activational effects of androgens contribute to sex differences in chirping in *A. leptorhynchus*, but likely explain only part of the sex difference; and (2) the androgenic regulation of within-sex variation in chirping in males is complex and may depend on social or environmental conditions.

The effects of androgen manipulations on chirping in other apteronotid species are consistent with the sexual dimorphism of chirping in those species. 11KT does not affect chirp rate in *A. albifrons*, which is sexually monomorphic for chirp rate (Dunlap et al., 1998; Ho et al., 2013). In *P. hasemani*, males produce longer duration chirps than females, and blocking androgen receptors with flutamide demasculinizes chirp duration in males (Petzold and Smith, 2012a). The role of estrogens in regulating chirping has not been studied in any apteronotid species.

Neural control of chirping

Just as EODf is controlled by a dedicated brain region (the PMN), chirping is controlled by a single brain nucleus, the thalamic prepacemaker nucleus (PPn; Fig. 1). Chirps are produced when projection neurons in the 'chirp' subdivision of the PPN (PPn-C) fire synchronously and their terminals in the PMN release glutamate (Dye, 1988; Dye and Heiligenberg, 1987; Kawasaki et al., 1988). Glutamatergic activation of kainite/quisqualate receptors on relay cells transiently increases their firing rates, which is translated into the abrupt increase in EODf that constitutes a chirp. When pacemaker, relay and electromotor neurons are excited strongly and fire at rates far above their baseline, voltage-gated sodium channels may inactivate and/or neuronal activity may desynchronize, which decreases the action potential amplitude and reduces EOD amplitude during high-frequency chirps (Dye, 1988).

Two hypotheses have been proposed for the mechanisms underlying the two different chirp types in *A. leptorhynchus*. One possibility is that low-frequency and high-frequency chirps are both produced by activation of PPn-C projection neurons, but that they result from different levels of recruitment. Recruitment of a subset of PPn-C neurons may result in the moderate increases in PMN firing rates and EODf characteristic of low-frequency chirps. At higher levels of PPn-C activation, coupling may result in large-scale recruitment of most or all projection neurons, resulting in intense excitation of the PMN and large increases in EODf characteristic of high-frequency chirps (Kawasaki et al., 1988). This hypothesis is supported by the finding that iontophoresis of substance P into the PPn both stimulates chirp production and increases their intensity, which suggests that the PPn may be a site initiating high-frequency as well as low-frequency chirps (Weld et al., 1991). An alternative possibility is that the PPn-C might control only low-frequency chirps, and that high-frequency chirps might be regulated by the sublemniscal prepacemaker nucleus (SPPn), a midbrain nucleus that also provides glutamatergic excitation to relay neurons in the PMN and that mediates the jamming avoidance response. Strong electrical stimulation of the SPPn produces interruption-like modulations of the EOD with large frequency increases and amplitude decreases that resemble high-frequency chirps (Heiligenberg et al., 1996).

Neuromodulation of chirping

The PPn is innervated richly by terminals containing numerous neuromodulators, including dopamine, noradrenaline (norepinephrine), serotonin, somatostatin, galanin, substance P and metenkephalin (reviewed in Zupanc and Maler, 1997). Although the function of most of these neuromodulators in the PPn is not known, two of them, serotonin and substance P, affect chirping and have sexually dimorphic expression.

Serotonin-immunoreactive terminals are more abundant in the PPn in females than in males (Telgkamp et al., 2007). Furthermore, fish with higher EODfs, which may be an indicator of dominance (see above), have reduced expression of serotonin in the PPn than fish with lower EODfs. This suggests that serotonin might both influence sex differences in chirping and link social rank and chirping. Intracerebroventricular injections of serotonin reduce chirping, which is consistent with the hypothesis that stronger serotonergic tone in females and subordinates may reduce their chirping rates (but see 'Function of chirps' below). Peripheral injections of specific serotonin receptor agonists and antagonists suggest that serotonergic regulation of chirping is complex. Agonists of $5HT_2$ receptors reduce chirp rates similar to intracerebroventricular injections of serotonin. Agonists of $5HT_{1A}$ receptors, in contrast, strongly increase chirp rates and the production of high-frequency chirps (Smith and Combs, 2008). Because the studies on the effects of serotonin and receptor agonists/antagonists did not use local applications of drugs to the PPn, their effects may have been mediated indirectly. For example, serotonin could influence other neuromodulatory inputs to the PPn or the sensory processing of the conspecific EOD stimuli that elicit

chirps (Deemyad et al., 2011). Further studies are needed to identify how serotonin directly and indirectly influences the excitability of PPn-C neurons, and the relationship between social rank/experience, serotonin and chirping.

The expression of substance P in the PPn is highly sexually dimorphic. Substance P-immunoreactive fibers are abundant in the PPn of male *A. leptorhynchus*, but nearly absent in the PPn of females (Kolodziejski et al., 2005; Weld and Maler, 1992). Local iontophoresis of substance P into the PPn stimulates chirping, and the substance P-induced chirps have greater duration and/or FM than glutamate-evoked chirps (Weld et al., 1991). Treatment of female *A. leptorhynchus* with androgens increases expression of substance P in the PPn and masculinizes chirp rate and structure (Dulka et al., 1995). These results suggest that androgens may increase chirp rate and/or masculinize chirp structure by increasing the expression and release of substance P in the PPn, which increases excitability and/or coupling of PPn-C neurons. The expression of substance P is also highly sexually dimorphic in *A. albifrons*, despite the fact that chirp rate is sexually monomorphic in this species (Kolodziejski et al., 2005). Male *A. albifrons*, like *A. leptorhynchus*, however, produce more high-frequency chirps than females; and male *A. albifrons* chirps are longer in duration than those of females (Fig. 2) (Dunlap and Larkins-Ford, 2003a; Kolodziejski et al., 2005). These findings suggest that substance P may regulate sex differences in chirp structure across apteronotid species, but that sexually dimorphic expression of substance P in the PPn is not sufficient for sex differences in chirp rate.

Arginine vasotocin (AVT) also modulates chirping behavior. AVT (and its homolog arginine vasopressin in mammals) is a powerful regulator of social behavior in vertebrates and often has more pronounced effects in males than in females (Godwin and Thompson, 2012). Peripheral injections of AVT increase the production of high-frequency chirps and reduce the production of low-frequency chirps in male *A. leptorhynchus*, but not in females (Bastian et al., 2001). If high-frequency chirps function as courtship signals (see 'Function of chirps' below), AVT may shift male behavior away from agonistic communication and toward reproductive communication. Unlike substance P and serotonin, however, AVT is not expressed in significant quantities in the PPn (B. Bernatowicz and G.T.S., unpublished observations), so its effects on chirping may be indirect.

Function of chirps

Chirps are produced primarily during social interactions. The function of chirping has been studied most extensively in *A. leptorhynchus*. Early studies of chirping suggested that low-frequency chirps function as aggressive signals, whereas high-frequency chirps function as courtship signals. More recent studies have provided some conflicting evidence suggesting that the signaling function of chirps may be more complex.

Several lines of evidence support the hypothesis that low-frequency chirps are aggressive signals in *A. leptorhynchus*. First, sex differences in the production of low-frequency chirps coincide with sex differences in aggression: most studies have found that males chirp more, and males are more aggressive than females (Dunlap, 2002; Hupé and Lewis, 2008; Triefenbach and Zakon, 2008) (but see Bohorquez and Smith, 2011). Second, low-frequency chirps are elicited most robustly by EODs or playbacks with frequencies within 5–20 Hz of the chirper. Thus, low-frequency chirps are most often directed towards individuals of the same sex, and towards individuals with similar EODfs. If EODf is a signal of social rank, low-frequency chirps are thus produced more often between individuals of similar ranks that are competing more intensely for resources. Finally, body size, 11KT levels and chirping during dyadic interactions are positively correlated in males (Dunlap, 2002) (but see Bohorquez and Smith, 2011; Cuddy et al., 2012). Furthermore, dominant males chirped more than subordinate males after (but not before) winning a conflict with another male (Triefenbach and Zakon, 2008). These findings suggest that larger, dominant males are more likely to chirp than smaller males.

Other studies, however, suggest that low-frequency chirps might signal submission or appeasement. Two studies of chirping during live male–male interactions found that fish chirped less around the time of an attack than several seconds before or after the attack (Hupé and Lewis, 2008; Triefenbach and Zakon, 2008). This finding suggests either that chirps are a submissive signal or that chirps are effective deterrents of escalated aggression. In a study of male *A. leptorhynchus* housed socially and reproductively stimulated by lowering water conductivity, the production of low-frequency chirps was not correlated with body size, EODf or 11KT levels across all males in the study, and in a subset of males with high EODfs, production of low-frequency chirps correlated negatively with EODf (Cuddy et al., 2012). In another study, the number of low-frequency chirps produced by males in live, dyadic interactions was not correlated with EODf (Hupé and Lewis, 2008). Furthermore, although males chirped at higher overall rates towards males whose EODfs were close to their own, they were more likely to echo the chirps of males with EODfs that differed more from their own (Hupé et al., 2008). In a study of males and females housed in small social groups for 1 week and then tested in dyadic interactions, dominant and subordinate males chirped at similar rates, and subordinate females chirped much more often than dominant females (Bohorquez and Smith, 2011). Furthermore, dominant fish did not chirp significantly more when they attacked, but subordinate fish chirped more right after being attacked. These conflicting findings suggest that the function of low-frequency chirps as aggressive *versus* subordinate signals may depend on both social context and the condition of the signaler. This complexity is further supported by the effects of chirp playbacks on aggressive behavior. Chirp playbacks reduce aggressive approaches towards decoys playing chirps with random timing, but do not affect aggressive approaches when delivered with a timing that echoes the chirps of the focal fish (Hupé, 2012; Walz et al., 2013).

High-frequency chirps were first identified as signals produced by male *A. leptorhynchus* during courtship and spawning (Hagedorn and Heiligenberg, 1985). Additional evidence that these chirps may function in courtship comes from playback studies showing that females very rarely produce high-frequency chirps, and that males produce more high-frequency chirps in response to playbacks of EODfs more than 100 Hz away from their own (Bastian et al., 2001; Cuddy et al., 2012; Kolodziejski et al., 2007; Triefenbach and Zakon, 2003). Thus, EODs of females, which are typically 100–300 Hz lower in frequency than those of males, are more likely to elicit high-frequency chirps from a male than are the EODs of most other males. However, males also produce high-frequency chirps in response to playbacks with frequencies 100–200 Hz higher than their own. Thus, although some evidence supports the hypothesis that high-frequency chirps may be directed preferentially by males to fish with much lower EODfs (i.e. females), they may also be produced to fish with much higher EODfs (e.g. by males with low EODfs to males with very high EODfs).

Relationships between androgens, dominance, EODf and the production of high-frequency chirps also provide mixed evidence

on their function. In live dyadic trials between males, the less aggressive fish produced more high-frequency chirps than the more aggressive fish, supporting the possible role of these chirps as intrasexual signals of subordinance (Hupé, 2012). Furthermore, stimulating gonadal recrudescence by lowering water conductivity did not increase high-frequency chirping, and the production of high-frequency chirps was negatively correlated with 11-KT concentrations in non-breeding males (Cuddy et al., 2012).

These contrasting findings suggest an interesting hypothesis: that high-frequency chirps might serve a dual function as both a courtship signal during spawning and a signal of submission in male–male interactions. Immature or subordinate males with low EODfs may produce high-frequency chirps when interacting with dominant males outside of the breeding season, which would explain chirping by subordinates and non-reproductive males in response to EODfs 100–200 Hz higher than their own. Reproductively active males may produce high-frequency chirps in response to females during courtship, which would explain the enhanced high-frequency chirping in response to 100–200 Hz lower EODfs and Hagedorn and Heiligenberg's observations of high-frequency chirping during spawning (Hagedorn and Heiligenberg, 1985). Additional recordings of high-frequency chirping behavior during spawning and live agonistic interactions are needed to test this hypothesis.

Many other apteronotid species also produce high-frequency chirps (i.e. chirps with >150 Hz FM and substantial AM), but they show different patterns in the context in which they produce those chirps. Unlike in *A. leptorhynchus*, high-frequency chirps in *A. albifrons* are produced by both males and female in response to EODfs close to the fish's own (i.e. to same-sex fish) (Kolodziejski et al., 2007). In some species with sexually monomorphic EODs (e.g. *A. bonapartii*), high-frequency chirps are produced by both sexes in response to a broad range of EODfs (Ho et al., 2010). This suggests that the social context and function of chirp types have also evolved.

Social context and sensory encoding of chirps

The evolution of chirp function may be linked to the mechanisms by which chirps are encoded by electrosensory systems in different social contexts. Wave-type electric fish detect each other's EODs *via* the beating amplitude envelope created when the EODs of two fish interfere constructively and destructively. The beat frequency is equal to the difference frequency (dF) between the two EODs: fish with similar EODfs (e.g. two fish of the same sex) produce slow beats; and fish with dissimilar EODfs (e.g. male–female pairs) produce fast beats. Beats are encoded by P-type tuberous electroreceptors (P-units), whose firing probability encodes EOD amplitude, and by pyramidal neurons in the electrosensory lateral line lobe (ELL) (reviewed in Marsat et al., 2012; Walz et al., 2013). Chirps disrupt beating patterns both because the difference between the two fish's EODfs (and thus beat frequency) changes abruptly and because reductions in EOD amplitude during high-frequency chirps reduce beat contrast (Benda et al., 2006). The effect of chirps on the amplitude envelope and on the resultant P-unit activity depends on the amount of chirp AM and FM and on the magnitude and sign of the dF (Benda et al., 2006; Walz et al., 2013).

In *A. leptorhynchus*, low-frequency chirps that are produced on the background of slow beats (i.e. dFs of <30 Hz) produce a robust phase shift in the beat cycle and strongly synchronize P-unit activity (Benda et al., 2006; Walz et al., 2013). These chirps, however, do not synchronize P-unit activity when they occur on the background of faster beats (i.e. large dFs). Thus, low-frequency chirps are most conspicuous to P-type electroreceptors when they are produced in the social context of two interacting fish with nearby EODfs.

Unlike low-frequency chirps produced in response to small dFs, which increase P-unit synchronization, high-frequency chirps often desynchronize P-unit firing. This desynchronization results both because high-frequency chirps increase dF and beat frequency by hundreds of Hz (outside the range of AM frequencies to which P-units synchronize) and because the decrease in EOD amplitude during high-frequency chirps reduces beat contrast. P-unit desynchronization caused by high-frequency chirps is most pronounced at larger dFs (i.e. ~50–200 Hz) (Benda et al., 2006). Thus, high-frequency chirps produce the most conspicuous change in P-unit firing patterns when they occur on the background of a relatively dissimilar EODf.

High-frequency chirps are also encoded differently than low-frequency chirps in the ELL (Marsat et al., 2012). Low-frequency chirps produce synchronous and highly stereotyped bursts in E-type pyramidal cells in the lateral segment of the ELL. I-type pyramidal cells, rather than E-type pyramidal cells, most robustly change their firing patterns during high-frequency chirps. Furthermore, unlike the responses of E-type cells to low-frequency chirps, the responses of I-type cells to high-frequency chirps are heterogeneous, which allows populations of these cells to encode variation in high-frequency chirp properties.

Differences in how low-frequency *versus* high-frequency chirps are encoded by the peripheral and central electrosensory system reinforces the hypothesis that these chirp types are distinct signals with potentially different communicative functions. Furthermore, differential effects of dF on the ability of P-units to encode these two chirp types suggest mechanistic and evolutionary linkages between sensory systems and the structure, function and social context of chirps. The fact that low-frequency chirps produce the most robust changes in P-unit firing synchrony when they occur on a background of low dFs makes them well adapted to serve as agonistic signals between rivals with similar EODfs (i.e. between same-sex individuals of similar rank). Similarly, the conspicuousness of high-frequency chirps on a background of large dFs makes these chirps well adapted to serve either as courtship signals (male EODf>>female EODf) or as signals between highly dominant (high EODf) and subordinate (low EODf) males. These relationships imply that sensory mechanisms may have evolved to detect these chirp types optimally in appropriate social contexts and/or that the structure and function of chirp types evolved to exploit pre-existing sensory mechanisms.

Conclusions and future directions

Comparative studies and the evolution of chirping and EODs

Much more is known about sex differences, hormonal control and the function of EODs and chirping in *A. leptorhynchus* (and to a lesser extent *A. albifrons*) than in other apterontid species. The little that is known about sex differences in electrocommunication in other apteronotid species suggests rapid divergent and convergent evolution.

EODs, chirping and their sexual dimorphism have diverged in several closely related taxa. Chirp structure and distinct chirp types differ markedly across *Adontosternarchus* species. *Adontosternarchus devenanzii* produces simple (single-peaked) chirps and complex (multi-peaked) chirps, and the production of those chirp types is sexually dimorphic (Zhou and Smith, 2006). All chirps in *A. balaenops* are single peaked, but unlike *A. devenanzii*, this species produces distinct high-frequency and low-frequency chirps (Turner et al., 2007). In both *P. hasemani* and *P.*

gimbelli, populations from the upper *versus* lower Amazon differ in chirp structure (Turner et al., 2007). Most chirps produced by Brazilian populations of *P. hasemani* and *P. gimbelli* have extreme AM, resulting in an EOD interruption, whereas Peruvian individuals of these species also produce chirps that do not interrupt the EOD. Sexual dimorphism also varies across closely related species. The genus *Apteronotus* contains examples of every pattern of sexual dimorphism in EODf: males>females in *A. leptorhynchus*; males<females in *A. albifrons*; and no sex difference in *A. magdalensis* (Dunlap et al., 1998; Maldonado-Ocampo et al., 2011). Furthermore, Orinoco and Amazonian populations of *A. albifrons* differ in the magnitude of sex differences in EODf (Ho et al., 2013).

Electrocommunication signals have also convergently evolved in several more distantly related apteronotid taxa. Triphasic EOD waveforms have evolved in three apteronotid lineages (Table 1; *Sternarchorhynchus*, *Magosternarchus*/*Sternarchella* and *Apteronotus* n. sp. B). Distinct chirp types based on certain chirp parameters have evolved in different genera (e.g. interruptions in *P. hasemani* and *P. gimbeli*; and single- and multi-peaked chirp types in *A. bonapartii* and *A. balaenops*). The numerous examples of convergence and divergence in EODs and chirping provide additional evidence that these signals evolve rapidly. Exploiting the diversity of electrical signals in ghost knifefishes to understand how sexually dimorphic communication signals and their underlying mechanisms evolve will require data on these signals and their hormonal and neural regulation in additional species. Such studies would also be facilitated by the development of a robust species-level molecular phylogeny of the Apterontidae and by the use of phylogenetically based comparative methods (Garland et al., 2005; Martins and Hansen, 1997).

Naturalistic studies on the function of EODs and chirps

Numerous studies of EODs and chirping in response to playbacks or during staged social interactions in *A. leptorhynchus* have yielded insight into the function of these signals. However, variations in experimental design sometimes have led to inconsistent results that suggest that the meanings of EODs and chirps depend on the condition of the signaler and/or social context. Additional studies, including studies of interacting fish under more naturalistic conditions (e.g. field studies, mate choice studies, studies of signaling in courting and spawning fish, etc.) are needed to clarify how these signals are used and how receivers respond to them in ecologically relevant contexts. Comparative studies of chirp function and social ecology in other apteronotid species may also provide additional insight by addressing how the function of electrocommunication signals has co-evolved with social structure and sexual dimorphism.

Molecular and cellular mechanisms regulating chirps and EODs

The electrocommunication signals of electric fish are well suited for studying how the physiological mechanisms regulating sexually dimorphic behavior evolve because EODf and chirping are responsive to hormones and are controlled by relatively simple and well-characterized neural circuits. Sexual dimorphism in EODf is well-correlated across species with the effects of androgens and/or estrogens on EODf, and androgens and estrogens change EODf by altering the firing rates of EMNs and neurons in the PMN. A possible mechanism by which species diversity in the sexual dimorphism of EODf has evolved is through changes in the expression of steroid-metabolizing enzymes or receptors in the PMN and EMNs, or through changes in the downstream targets of these receptors. Comparatively examining the sequence, expression patterns and molecular targets of androgen receptors, estrogen receptors and aromatase in the electromotor systems of apteronotid species that differ in sexual dimorphism of EODf can test this hypothesis and potentially reveal the molecular mechanisms underlying the evolution of sex differences.

Just as EODf is controlled by a dedicated brain nucleus (the PMN), chirping is also controlled by a single brain area (the PPn-C). The regulation of chirping, however, is more complex than that of EODf, and involves not only steroidal regulation of sex differences but also numerous neuromodulators. The PPn-C is richly innervated by neuropeptides and biogenic amines and receives input from the nucleus electrosensorius, the optic tectum, the anterior hypothalamus/preoptic area and the preglomerular nucleus, which may relay inputs from hypothalamic and telencephalic areas (Zupanc, 2002). These pathways provide numerous control points to regulate chirping, including the intrinsic excitability and connectivity of PPn-C neurons, the robustness and efficacy of neuromodulatory inputs to the PPn-C, and the strength and nature of inputs to the PPn-C from the electrosensory system. Our understanding of how diverse chirp types are produced by the PPn-C is rudimentary and would be facilitated by studies addressing several questions. (1) How are firing patterns of PPn-C neurons related to chirp structure? (2) Are species and sex differences in chirp structure caused by differences in PPn-C neuron activity, by differences in the response of PMN neurons to PPn-C input, or both? (3) What are the molecular and cellular mechanisms by which gonadal steroid hormones and neuromodulators change the excitability, connectivity or extrinsic inputs of PPn-C neurons to modulate chirp rate and/or structure?

Coordinated regulation of communication signals and sensory systems

One of the most exciting developments in the study of electrocommunication has been an increased understanding of how the electrosensory system extracts information from electrocommunication signals and the importance of social context in signal coding (Chacron et al., 2011; Marsat et al., 2012; Walz et al., 2013). This work has built a foundation for exciting future studies on how hormones and neuromodulators coordinately regulate the reception and production of communication signals. For example, androgens not only regulate EODf and chirping but also change the tuning of tuberous electroreceptors (Meyer et al., 1987). Additional studies are needed to determine whether androgens or estrogens also influence how the central electrosensory system processes electrocommunication signals.

Neuromodulators also provide a powerful mechanism to coordinate signal production and reception. Serotonin, for example, is expressed in both the PPn and the lateral segment of the ELL, which is particularly involved in processing electrocommunication signals (Johnston et al., 1990; Telgkamp et al., 2007). Serotonin both modulates chirp production and changes the way that ELL pyramidal neurons encode communication signals, specifically by enhancing their ability to encode both low-frequency beats created by same-sex interactions and the low-frequency chirps produced during such interactions (Deemyad et al., 2011). These findings suggest that serotonin might serve as a mediator for animals to coordinate signal production and reception in response to their condition and/or short-term and long-term social experiences. Further studies of the stimuli that elicit serotonin release in the ELL and PPn and on the role of other neuromodulators in regulating chirp production and detection will help test this hypothesis.

List of abbreviations

AM	amplitude modulation
AVT	arginine vasotocin
dF	difference frequency
ELL	electrosensory lateral line lobe
EMN	electromotor neuron
EOD	electric organ discharge
EODf	EOD frequency
FM	frequency modulation
PMN	pacemaker nucleus
PPn	prepacemaker nucleus
PPn-C	chirp subdivision of the PPn
SPPn	sublemniscal PPn

Competing interests

No competing interests declared.

Funding

This work was supported by the National Science Foundation [grant no. IOS 0950721].

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