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Divergence in androgen sensitivity contributes to population differences in sexual dimorphism of electrocommunication behavior

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Abstract

Weakly-electric fish (Apteronotidae) produce highly diverse electrocommunication signals. Electric organ discharges (EODs) vary across species, sexes, and in the magnitude and direction of their sexual dimorphism. Gonadal steroid hormones can modulate EODs, and differences in androgen sensitivity are hypothesized to underlie variation in the degree of sexual dimorphism across species. In this study, we asked whether variation in androgen sensitivity explained variation in sexual dimorphism of EODs within species, at the population level. We examined two populations of black ghost knifefish (*Apteronotus albifrons*), one from the Orinoco and the other from the Amazon river basin. EOD frequency (EODf) and chirp rates were measured to characterize diversity in sexual dimorphism across populations. The magnitude of sexual dimorphism in EODf differed significantly across populations, and was more pronounced in the Orinoco population than in the Amazon population. Chirp rates were sexually monomorphic in both populations. 11-ketotestosterone (11-kT) was administered over a two-week period to assess population differences in sensitivity to androgens. 11-kT masculinized EODf significantly more in the population with the greater degree of sexual dimorphism. 11-kT had no effect on the sexually monomorphic chirping rates. We conclude that population divergence in androgen sensitivity contributes to variation in sexual dimorphism of EODf in *A. albifrons*.

Keywords

Apteronotus albifrons; behavior; black ghost knifefish; chirping; communication; electric fish; EOD; 11-ketotestosterone; population; sexual dimorphism

Introduction

Animal communication, particularly in the context of reproduction, is often sexually dimorphic. The magnitude and direction of sexual dimorphism in communication signals can vary across species (e.g. Brenowitz 1997; Wiens et al. 1999; Johnson and Wade 2010),

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and populations (e.g. Hill 1994; Ritchie et al. 2007; Maan and Cummings 2009). Variation in sexually dimorphic behavior can result from changes in underlying physiology, and attempts to understand the origins of sexual dimorphism have led to comparisons of the neuroendocrine basis of sex differences across species (e.g. Brenowitz et al., 1985; Moore, 1991; Hews et al., 2012). Comparative studies of variation in the magnitude and direction of sexual dimorphism, particularly at the population level, present an opportunity to integrate neuroendocrine physiology with evolutionary biology. Nevertheless, such studies remain uncommon, as are examples of variation in sexually dimorphic behavior at the population level.

The Apterontidae (ghost knifefish) are a highly derived and speciose family of South American weakly-electric fish (Mago-Leccia, 1994; Alves-Gomes, 1995; Crampton and Albert, 2006). They produce diverse electrocommunication behaviors that vary across sexes and species, making them an attractive system for understanding the proximate causes of sexual dimorphism in behavior (Turner et al., 2007). Apterontids generate electric organ discharges (EODs) – continuous, quasi-sinusoidal signals with very regular frequencies (EODf). During social interactions, fish produce brief modulations of the EODf called chirps. Both EODf and chirps function as reproductive and agonistic communication signals (Hagedorn and Heiligenberg, 1985; Zakon and Smith, 2009).

EODf and chirping behavior are sensitive to gonadal steroids in several species of electric fish. Estradiol (E2) feminizes EODf (Meyer, 1987; Dunlap, 1997), whereas the non-aromatizable androgens 11-ketotestosterone (11-kT) and 5 α -DHT masculinize EODf (Schaefer and Zakon, 1996; Dunlap and Zakon, 1998). Variation in hormone sensitivity has been hypothesized to underlie species differences in the magnitude and direction of sexual dimorphism in EOD behavior (Dunlap et al., 1998). For example, although males have higher EODfs than females in brown ghosts (*Apterontus leptorhynchus*), the direction of sexual dimorphism is reversed in the closely-related black ghosts (*Apterontus albifrons*). This reversal is accompanied by a corresponding change in endocrine regulation, whereby androgens masculinize behavior by raising EODf in brown ghosts, but decrease EODf in black ghosts (Dunlap et al., 1998). In this study, we examined the sexual dimorphism of electrocommunication signals in different populations of black ghost knifefish (*Apterontus albifrons*) to investigate the extent of population-level variation in sexual dimorphism. We used hormone treatments to ask whether and how androgen sensitivity plays a role in generating diversity in sexually dimorphic behavior.

Methods

Animals

Black ghost populations from the Brazilian Amazon (abbreviated as BR; male n=10 (mean $\pm$ SD body mass; 72.1 $\pm$ 17.7g), female n=15 (55.7 $\pm$ 11.7g)) and the Colombian Orinoco (abbreviated as CO; male n=6 (67.0 $\pm$ 8.7g), female n=5 (48.4 $\pm$ 6.2g)) were collected by commercial fish suppliers. Fish were brought to Indiana University and maintained in the laboratory within a 2000-l recirculating freshwater system on a 12:12 light/dark cycle. Fish were housed at low conductivity (50-300 μ S/cm), 25.5-27.5°C, pH 6.0-7.0. Animal care and experimental protocols were approved by the Indiana University Bloomington Institutional Animal Care and Use Committee.

Behavior

EODf was measured with a Fluke 187 multimeter (model 187; Everett, WA, USA), using a pair of wires placed next to the fish and amplified (gain 100X; Grass model P-55). Measurements were temperature compensated to 26°C using a Q10°C of 1.6 (Dunlap et al.

2000; Ho et al. 2010). Chirps were recorded by using a previously described playback paradigm (Turner et al. 2007). Individuals were held in perforated PVC tubes with plastic mesh at either end and placed in a darkened 38-l tank containing water from the fishes' home tank. Water temperature typically varied less than 0.2°C during the recording session. A pair of carbon electrodes was positioned at the head and tail of the fish to record the fish's own discharge, and another pair was placed on either side of the fish's tube to deliver playback stimuli. Fish were acclimated for 30 minutes before recordings.

Chirp Stimulus—Playback stimuli were sinusoidal voltage signals generated using Cool Edit Pro (Syntrillium; Phoenix, AZ, USA), played back through a sound card (Soundmax Digital Audio, Analog Devices, Inc.), and calibrated using a Fluke multimeter to a field-intensity of approximately 1.6mV/cm at a point midway between and collinear to the stimulus electrodes. This signal intensity approximates the EOD of a medium-sized conspecific. We split the stimulus signal, routing one copy into the experimental tank and digitizing the other on the right channel of the recording sound card (see below). Each fish was presented, in random order, with a series of five stimuli designed to cover the range of conspecific EOD frequencies: -150Hz, -20Hz, -5Hz, +20Hz, and +150Hz, relative to the fish's own EODf. After a baseline recording with no stimulus, each stimulus was presented as a four minute trial separated by ten minute intervals. Each trial included a one minute pre-stimulus period (stimulus off), two minutes of playback stimulus (stimulus on), and one minute of post-stimulus (stimulus off).

Chirp Recording—Signals from the recording electrodes were differentially amplified (gain 100-1000X; Grass model P-55), band-pass filtered (0.1 Hz – 10 kHz), and digitized at 44.1 kHz on the left channel of a sound card in a computer running Cool Edit Pro (Syntrillium, Phoenix, AZ, USA).

Chirp Analysis—EOD signals were analyzed offline using customized procedures (efish23e0, Brian Nelson, University of Oregon, Eugene, OR, <http://nelsonbs.com/eFish/efish.html>) running in Igor Pro as detailed in an earlier study (Kolodziejewski et al. 2005). Briefly, any contamination from the playback stimulus was removed by subtracting a copy of the playback, appropriately scaled and phase-shifted, from the recording. An autocorrelation algorithm with a 6ms Hanning window advanced 2 ms per iteration was used to determine the fundamental EOD frequency. Baseline frequency was defined as the mode frequency over a sliding 1s window. The procedure detected EOD modulations (EODms: chirps, rises) when a frequency elevation of 3Hz or greater over the baseline persisting for a duration of at least 10ms but less than 30s, and a minimum inter-EODm interval of 100ms. The beginning and end of each detected EODm was defined by the EODf coming within 1Hz of the baseline frequency. Duration and peak frequency parameters were automatically quantified for each EODm. Frequency traces were visually inspected following analysis to ensure accurate identification and quantification of EODms. Based on previous studies, EODms with $FM > 21 \times (\text{duration}) + 10\text{Hz}$ were classified as chirps (Turner et al. 2007).

Hormone treatment

11-ketotestosterone (11-kT) and control (DMSO) treatments were administered in a sequential design with treatment order randomized. Water conductivity was raised (>500µS/cm) to induce gonadal regression and minimize production of endogenous steroids. Females were fed using earthworms injected with only DMSO, or DMSO and 11-kT. A small amount of food dye (McCormick & Co., Inc.; 1 drop / 15mL) was added to the hormone and control solutions for visualization during earthworm preparation. Earthworms were fed to the fish every 12h, at a dose adjusted for each fish's body mass (0.06ug 11-kT / 1g body mass). Treatments were given over 14 days, with a minimum of four intervening weeks to

allow clearance of exogenous steroids. Changes in EODf were measured every two days over the course of each treatment. Chirp assays were performed at the end of the treatment period, between 3-5h after feeding.

Hormone assay

Blood samples were taken to verify plasma levels gonadal steroids, 11-ketotestosterone (11-kT) and estradiol (E2). Five to six hours after the final feeding of each treatment, blood was drawn from the hemal arch and centrifuged for five minutes to extract plasma. Plasma was stored at -20°C . Enzyme immunoassays (Cayman Chemical, Ann Arbor, MI) were performed according to the manufacturer's directions. Sensitivity thresholds were reported at 5pg/mL (11-kT) and 129pg/mL (E2). Plasma samples were diluted in assay buffer at concentrations of 1:25 or 1:500 (11-kT) and 1:12 (E2). Intra-assay variations were 10.6% and 17.2% respectively for each 11-kT assay, and 14.2% and 23.2% respectively for each E2 assay. Inter-assay variation was 2.4% for 11-kT and 10.9% for E2.

Statistics

Statistical analyses were performed with Statistica 7 (StatSoft Inc.; Tulsa, OK, USA). Sexual dimorphism in EOD and the effects of hormone treatment were analyzed using factorial ANOVAs. 11-kT and E2 levels were analyzed using a repeated measures analyses of variance (RMANOVA) with treatment and population as factors. Tukey's HSD was used to test for differences among treatments. Statistical significance was set at an alpha level of 0.05.

Genetic population markers

To determine whether fish originated from genetically differentiated populations, tissue samples were taken from both populations (BR, Rose Tropicals, Miami, FL; CO, Ornamental Fish Distributors, Miami, FL), as well as additional individuals from the Colombian Orinoco (CO-oo, n=12. Ruinemans Aquarium, Miami, FL), Peruvian Amazon (PE, n=5. Ornamental Amazon Fish Aquarium, Iquitos, Peru; Rio Nanay, Peru), and Colombian Amazon (CO-az, n=5). Total DNA was extracted from fin clips using a DNEasy kit (Qiagen Inc.) according to the manufacturer's instructions. We amplified a portion of the mitochondrial cytochrome-b (cyt-b) gene, a marker used extensively for population and species level analyses across vertebrates (Meyer 1994). Primers (L-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA, H-AAGGACGTATCCGACGAAGGCAGTTACTATTATTA) were designed based on previously described universal primers. PCR was performed in standard 50uL reactions under the following thermal cycling conditions: 1 min at 93°C ; 1 min at 50°C ; 2 min at 72°C , for 30 cycles. PCR products were purified using magnetic particles and sequenced in both directions to verify the validity of sequence data. Sequences were visualized using a BigDyeTM Terminator v3.1 cycle sequencing kit (Applied Biosystems) on an ABI3730 DNA automated sequencer (Applied Biosystems). Sequences were aligned using CodonCode Aligner 3.7.1.

Indices of genetic variation and population structure were calculated in Arlequin 3.5.1.2. (Excoffier and Lischer 2010). Genetic distances between populations were calculated using pairwise F_{st} , with significance determined using 1000 permutations. A hierarchical analysis of molecular variance (AMOVA) was used to explore population structure by partitioning variance across the Amazon and Orinoco drainages, across populations, and within populations. A minimum spanning network was computed and visualized using TCS v1.21 (Clement et al. 2000).

Results

Sexual dimorphism in EODf

EODf was sexually dimorphic (Factorial ANOVA; effect of sex $F(1,32)=37.47$, $p<0.0001$). The CO population had a higher overall EODf than the BR population (effect of population $F(1,32)=22.88$, $p<0.0001$), an effect driven by high EODf in CO females. Populations differed significantly in the magnitude of sexual dimorphism in EODf – although males had lower EODf than females in both populations (Tukey HSD, $p=0.014$ (BR), $p<0.001$ (CO)), the sex difference was significantly greater in the CO population (population by sex interaction $F(1,32)=6.19$, $p=0.018$) (Fig.1). Effect size was greater for the CO population ($d=6.5$) than for the BR population ($d=1.2$).

Sexual dimorphism in chirping

Unlike EODf, chirp rates did not differ between sexes (Factorial ANOVA; effect of sex $F(1,25)=2.54$, $p>0.1$), although there was a non-significant trend in the CO population for males to chirp more than females. The CO population chirped at greater rates than the BR population (effect of population; $F(1,25)=7.30$, $p=0.012$), but populations did not differ significantly in the magnitude of sexual dimorphism in chirp rates (population by sex interaction; $F(1,25)=3.42$, $p=0.076$) (Fig.2). Effect size was moderate for the CO population ($d=0.5$) and greater than that of the BR population ($d=0.1$).

Hormones

Populations did not differ in 11-kT levels (effect of population $F(1,9)=0.038$, $p>0.5$, nor in response to hormone treatment (population by treatment interaction $F(1,9)=0.038$, $p>0.5$). Females treated with 11-kT had significantly higher levels of circulating 11-kT than individuals treated with DMSO controls (RMANOVA; effect of treatment $F(1,9)=30.92$, $p<0.001$). Control females had levels near the detection threshold of 11-kT, whereas steroid treated females had significantly higher 11-kT levels (table 1). Circulating E2 decreased slightly in 11-kT treated animals (effect of treatment $F(1,9)=5.84$, $p=0.039$); there was no effect of population (effect of population $F(1,9)=0.15$, $p>0.5$, treatment by population interaction $F(1,9)=0.11$, $p>0.5$) (table 1).

Effects of androgen treatment on EODf

EODf decreased significantly in response to 11-kT, as compared to controls (Factorial ANOVA; effect of treatment $F(1,20)=34.78$, $p<0.00001$). However, the effect of 11-kT differed across populations. The decrease in EODf in the Orinoco population was approximately two-fold greater than in the Amazon population (population by treatment interaction $F(1,20)=5.86$, $p=0.025$) (Fig.3).

Effects of androgen treatment on chirping

11-kT did not significantly affect chirping, as compared to controls (Factorial ANOVA; effect of treatment $F(1,20)=3.20$, $p=0.089$) in either population (effect of population; treatment by population interaction $F(1,20)=0.018$, $p>0.5$; $F(1,20)=1.17$, $p>0.1$) (Fig.4).

Genetic population structure

A 307-bp region was amplified from the cyt-b gene of 40 individuals across five geographically distinct populations. 13 of 307 sites were variable, with a maximum pairwise divergence of 2.9% between haplotypes. We identified six unique haplotypes, four of which were restricted to single populations (CO-az, BR), and two others that were shared across multiple populations (PE and CO/CO-oo). Haplotype diversity was highest across individuals from the BR population; all other populations exhibited only one haplotype each.

Pairwise F_{st} comparisons revealed strong genetic differentiation ($F_{st} = 0.212 - 1.00$, $p < 0.05$) between all populations except those with shared haplotypes (i.e. BR and PE; CO-oo and CO) (see Suppl. Materials for haplotype map). We ran a hierarchical AMOVA to partition variance across drainages and populations. Among drainage variance accounted for 83.56%, among population variance accounted for 12.07%, and within population variation accounted for 4.37% of the total genetic variation. Thus, populations showed significant genetic structure across geographic locations, as well as a striking division in genetic variation between populations from the Amazon and Orinoco drainages.

Discussion

Black ghosts from the Orinoco were strongly sexually dimorphic in EODf. Consistent with previous studies that did not account for population, females had higher EODfs than males (Dunlap et al. 1998; Kolodziejski et al. 2005). We found however, that fish from the Amazon were significantly less sexually dimorphic in EODf. Previous studies of black ghosts from undefined populations reported variation in the degree of sexual dimorphism of EODf, from sexual monomorphism (Dunlap and Larkins-Ford, 2003) to moderate (Dunlap et al. 1998) or strong sexual dimorphism (Kolodziejski et al. 2005). These results are consistent with population differences in sexual dimorphism, as reported in our study.

Chirp rate however, was not significantly sexually dimorphic in either population, although the CO population tended to chirp more, driven by a higher chirp rate in CO males than females. While our ability to detect sex differences is limited by sample size, this result is consistent with previous studies showing sexual monomorphism in chirp rate of black ghosts (Dunlap and Larkins-Ford, 2003; Dunlap et al. 1998; Kolodziejski et al. 2005).

Population differences in the magnitude of sexual dimorphism in EODf were paralleled by population variation in androgen sensitivity. Specifically, black ghosts from the Orinoco population were more sexually dimorphic in EODf and were more strongly masculinized by 11-kT than black ghosts from the Amazon population (Fig.2). This result suggests that the evolution of sexual dimorphism in behavior at the population level is accompanied by changes in androgen sensitivity across populations. Diversity in androgen sensitivity across populations may have multiple causes, including the evolution of variation in the quantity or tissue-specific distribution of androgen receptors, in the activity of enzymes involved in androgen metabolism, or in downstream signaling cascades (e.g. Adkins-Regan, 2008). In other gymnotiform fish, there is evidence that temporal variation in behavior is related to seasonal changes in the expression of androgen receptors in the CNS (Pouso et al., 2010).

Increases in 11-kT during 11-kT treatment were accompanied by slight decreases in circulating E2. This suppression of E2 is consistent with negative feedback provided by exogenous steroids acting on the HPG axis (Soma et al. 1996), or from possible down-regulation of ovarian aromatase activity (Li et al., 2006). In the closely-related brown ghosts, EODf is influenced by both androgens and estrogens (Meyer et al., 1987; Dulka and Maler, 1994; Schaefer and Zakon, 1996). It is not known whether EODf is also sensitive to estrogens in black ghosts. Nevertheless, we cannot eliminate the possibility that variation in E2 sensitivity may also be involved in population differences in sexual dimorphism.

11-kT treatments masculinized EODf in females, indicating that activational effects of steroid hormones contribute to adult EODf. However, although 11-kT levels reached the upper ranges of physiological 11-kT reported in apteronotid males (Dunlap et al. 1998; Dunlap, 2002), androgen administration did not completely masculinize EODf and explained only about 30% of the sex difference in EODf from each population. The effects

of 11kT on adult fish likely work in conjunction with activational effects of other steroids, organizational effects, or other non-steroidal physiological mechanisms to regulate EODf.

Exact capture locations were difficult to obtain from commercial suppliers. Nevertheless, our sequence data indicated that the BR and CO fish were genetically distinct groups, clustering together with other putative populations from the Amazon and Orinoco, respectively. Observed levels of sequence divergence (2.9%) were concordant with *cyt-b* genetic distances between extant congeneric fish species (<12%) (Johns and Avise 1998). Animals in this study were identified as *Apteronotus albifrons* (Mago-leccia, 1994), although morphological data suggests that *A. albifrons* may comprise a species complex (de Santana, 2003). All individuals presented with two light bands on the caudal peduncle, a feature diagnostic of this species complex. Thus, the BR and CO fish likely represent, at the least, divergent populations, but may also be closely-related, cryptic species.

Overall, we found that changes in androgen sensitivity of EODf across black ghost populations paralleled changes observed at the species level. For example, the reversal in direction of sexual dimorphism in EODf across brown and black ghosts is accompanied by changes in androgen regulation. Furthermore, sexual monomorphism in EOD chirping behavior of black ghosts is accompanied by a corresponding loss in androgen sensitivity (Dunlap et al., 1998). The magnitude and direction of sexual dimorphism in *Apteronotids* appear to be exceptionally evolutionarily labile, and could be important in generating species diversity (Butler et al. 2007). Understanding the proximate bases of sexual dimorphism allows us to understand how endocrine physiology and sexually dimorphic behavior contribute emergent processes such as speciation and adaptive radiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Literature Cited

- Adkins-Regan E. Do hormonal control systems produce evolutionary inertia? *Phil. Trans. R. Soc. B.* 2008; 363:1599–1609. [PubMed: 18048293]
- Alves-gomes JA, Orti G, Haygood M, Heiligenberg W, Meyer A. Phylogenetic analysis of the south-american electric fishes (order gymnotiformes) and the evolution of their electrogenic system - a synthesis based on morphology, electrophysiology, and mitochondrial sequence data. *Molecular Biology and Evolution.* 1995; 12(2):298–318. [PubMed: 7700155]
- Brenowitz EA, Arnold AP, Levin RN. Neural Correlates of Female Song in Tropical Duetting Birds. *Brain Research.* 1985; 343(1):104–112. [PubMed: 4041847]
- Brenowitz EA. Comparative approaches to the avian song system. *Journal of Neurobiology.* 1997; 33:517–531. [PubMed: 9369457]
- Butler MA, Sawyer SA, Losos JB. Sexual dimorphism and adaptive radiation in *Anolis* lizards. *Nature.* 2007; 447:202–205. [PubMed: 17495925]
- Clement M, Posada D, Crandall K. TCS: a computer program to estimate gene genealogies. *Molecular Ecology.* 2000; 9:1657–1660. [PubMed: 11050560]

- Crampton, WGR.; Albert, JS. Evolution of electric signal diversity in gymnotiform fishes. In: Ladich, F.; Collin, SP.; Moller, P.; Kapoor, BG., editors. *Communication in Fishes*. Vol. 2. Science Publishers; Enfield, NH: 2006. p. 647-731.
- Dulka JG, Maler L. Testosterone modulates female chirping behavior in the weakly electric fish *Apteronotus leptorhynchus*. *Journal of Comparative Physiology A*. 1994; 174:331-343.
- Dunlap KD. Hormonal and body size correlates of electrocommunication behavior during dyadic interactions in a weakly electric fish, *Apteronotus leptorhynchus*. *Horm. Behav.* 2002; 41(2):187-194. [PubMed: 11855903]
- Dunlap KD, McAnelly ML, Zakon HH. Estrogen modifies an electrocommunication signal by altering the electrocyte sodium current in an electric fish, *Sternopygus*. *J. Neurosci.* 1997; 17(8):2869-2875. [PubMed: 9092608]
- Dunlap KD, Larkins-Ford J. Diversity in the structure of electrocommunication signals within a genus of electric fish, *Apteronotus*. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology*. 2003; 189:153-161.
- Dunlap KD, Thomas P, Zakon HH. Diversity of sexual dimorphism in electrocommunication signals and its androgen regulation in a genus of electric fish, *Apteronotus*. *J. Comp. Phys. A – Neuroethol. Sens. Neural Behav. Phys.* 1998; 183:77-86.
- Dunlap KD, Zakon HH. Behavioral actions of androgens and androgen receptor expression in the electrocommunication system of an electric fish, *Eigenmannia virescens*. *Horm. Behav.* 1998; 34:30-38. [PubMed: 9735226]
- Dunlap KD, Smith GT, Yekta A. Temperature dependence of electrocommunication signals and their underlying neural rhythms in the weakly electric fish, *Apteronotus leptorhynchus*. *Brain Behavior and Evolution*. 2000; 55:152-162.
- Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*. 2010; 10:564-567. [PubMed: 21565059]
- Hagedorn M, Heiligenberg W. Court and Spark - Electric signals in the courtship and mating of Gymnotoid fish. *Anim. Behav.* 1985; 33:254-265.
- Hews DK, Hara E, Anderson MC. Sex and species differences in plasma testosterone and in counts of androgen receptor-positive cells in key brain regions of *Sceloporus* lizard species that differ in aggression. *General and Comparative Endocrinology*. 2012; 176(3):493-499. [PubMed: 22230767]
- Hill GE. Geographic-variation in male ornamentation and female mate preference in the house finch - a comparative test of models of sexual selection. *Behavioral Ecology*. 1994; 5:64-73.
- Ho WW, Fernandes CC, Alves-Gomes JA, Smith GT. Sex differences in the electrocommunication signals of the electric fish *Apteronotus bonapartii*. *Ethology*. 2010; 116:1050-1064. [PubMed: 20953311]
- Johns GC, Avise JC. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. *Molecular Biology and Evolution*. 1998; 15:1481-1490. [PubMed: 12572611]
- Johnson MA, Wade J. Behavioural display systems across nine *Anolis* lizard species: sexual dimorphisms in structure and function. *Proceedings of the Royal Society B-Biological Sciences*. 2010; 277(1688):1711-1719.
- Kolodziejski JA, Nelson BS, Smith GT. Sex and species differences in neuromodulatory input to a premotor nucleus: A comparative study of substance P and communication behavior in weakly electric fish. *J. Neurobiol.* 2005; 62(3):299-315. [PubMed: 15515000]
- Li G-L, Liu X-C, Lin H-R. Effects of aromatizable and nonaromatizable androgens on the sex inversion of red-spotted grouper (*Epinephelus akaara*). *Fish Physiology and Biochemistry*. 2006; 32(1):25-33. [PubMed: 20035475]
- Maan ME, Cummings ME. Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:19072-19077. [PubMed: 19858491]
- Mago-Leccia, F. Biblioteca de la academia de ciencias físicas, matemáticas y naturales. Vol. vol. XXIX. Fudeci, Caracas, Venezuela: Electric fishes of the continental waters of America.

- Meyer A. Shortcomings of the cytochrome-b gene as a molecular marker. *Trends in Ecology & Evolution*. 1994; 9:278–280. [PubMed: 21236853]
- Meyer JH, Leong M, Keller CH. Hormone-induced and maturational changes in electric organ discharges and electroreceptor tuning in the weakly electric fish *Apteronotus*. *J. Comp. Physiol. A- Sensory Neural and Behavioral Physiology*. 1987; 160(3):385–394.
- Moore MC. Application of organization-activation theory to alternative male reproductive strategies: a review. *Horm. Behav.* 1991; 25:154–179. [PubMed: 2066078]
- Pouso P, Quintana L, Bolatto C, Silva AC. Brain androgen receptor expression correlates with seasonal changes in the behavior of a weakly electric fish, *Brachyhypopomus gauderio*. *Horm. Behav.* 2010; 58:729–736. [PubMed: 20688071]
- Ritchie MG, Hamill RM, Graves JA, Magurran AE, Webb SA, Garcia CM. Sex and differentiation: population genetic divergence and sexual dimorphism in Mexican goodeid fish. *Journal of Evolutionary Biology*. 2007; 20:2048–2055. [PubMed: 17714321]
- de Santana CD. *Apteronotus caudimaculosus* n. sp. (Gymnotiformes: Apteronotidae), a sexually dimorphic black ghost knifefish from the Pantanal, Western Brazil, with a note on the monophyly of the *A. albifrons* species complex. *Zootaxa*. 2003; 252:1–11.
- Schaefer JE, Zakon HH. Opposing actions of androgen and estrogen on in vitro firing frequency of neuronal oscillators in the electromotor system. *J. Neurosci.* 1996; 16(8):2860–2868. [PubMed: 8786459]
- Soma KK, Francis RC, Wingfield JC, Fernald RD. Androgen regulation of hypothalamic neurons containing gonadotropin-releasing hormone in a cichlid fish: Integration with social cues. *Horm. Behav.* 1996; 30(3):216–226. [PubMed: 8918677]
- Turner CR, Derylo M, de Santana CD, Alves-Gomes JA, Smith GT. Phylogenetic comparative analysis of electric communication signals in ghost knifefishes (Gymnotiformes : Apteronotidae). *Journal of Experimental Biology*. 2007; 210(23):4104–4122. [PubMed: 18025011]
- Wiens JJ, Reeder TW, De Oca ANM. Molecular phylogenetics and evolution of sexual dichromatism among populations of the Yarrow's spiny lizard (*Sceloporus jarrovi*). *Evolution*. 1999; 53:1884–1897.
- Zakon, HH.; Smith, GT. Weakly-electric fish: behavior, neurobiology, and neuroendocrinology. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. 2nd edn. Vol. vol. 1. Academic Press; San Diego: 2009. p. 611-638.

Highlights

- We examined diversity in behavior across populations of black ghost knifefish.
- Populations varied in magnitude of sexual dimorphism in EOD frequency.
- Chirp rates were not significantly sexually dimorphic in either population.
- Differences in androgen sensitivity contribute to diversity in sexual dimorphism.

Fig.1. EODf in male (gray bars) and female (black bars) black ghosts from Orinoco and Amazon populations. The magnitude of sexual dimorphism in EODf was greater in the Orinoco population than in the Amazon population (mean $\pm$ SE; * p <0.05, *** p <0.001).

Fig.2. Number of chirps produced by male (gray bars) and female (black bars) black ghosts from Orinoco and Amazon populations. Chirp rates were sexually monomorphic, although populations differed overall in chirp production (mean $\pm$ SE; * $p < 0.05$).

Fig.3. Changes in EODf following control (DMSO) and 11-kT treatments of female black ghosts from the Amazon (black triangle) and Orinoco (gray circle) populations. EODf decreased more in response to 11-kT in the Orinoco population than in the Amazon population (mean $\pm$ SE; * $p < 0.05$).

Fig.4. Changes in chirp production following control (DMSO) and 11-kT treatments of female black ghosts from the Amazon (black triangle) and Orinoco (gray circle) populations. Chirping was not significantly affected by 11-kT in either population (mean $\pm$ SE).

Table 1

Circulating concentrations of 11-ketotestosterone (11-kT) and estradiol (E2) following DMSO and 11-kT treatments (mean $\pm$ SE)

<i>Treatment</i>	11-kT (ng/mL)^b		E2 (pg/mL)^a	
	<i>Orinoco</i>	<i>Amazon</i>	<i>Orinoco</i>	<i>Amazon</i>
DMSO	0.05 $\pm$ 0.007	0.05 $\pm$ 0.006	246.0 $\pm$ 56.4	216.6 $\pm$ 42.6
11-kT	11.6 $\pm$ 3.2	10.8 $\pm$ 2.4	165.0 $\pm$ 35.4	155.0 $\pm$ 26.8

^a p<0.05; significant difference in steroid levels between 11-kT and DMSO treated fish.

^b p<0.001; significant difference in steroid levels between 11-kT and DMSO treated fish.