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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*.

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; Johnson and Wade, 2010; Wiens et al., 1999), and populations (e.g. Hill, 1994; Maan and Cummings, 2009; Ritchie et al., 2007). 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; Hews et al., 2012; Moore, 1991). 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 Apteronotidae (ghost knifefish) are a highly derived and speciose family of South American weakly-electric fish (Alves-Gomes et al., 1995; Crampton and Albert, 2006; Mago-Leccia, 1994). 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). Apteronotids 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 (Dunlap et al., 1997; Meyer et al., 1987), whereas the non-aromatizable androgens 11-ketotestosterone (11-kT) and 5α-DHT masculinize EODf (Dunlap and Zakon, 1998; Schaefer and Zakon, 1996). 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 (*Apteronotus leptorhynchus*), the direction of sexual dimorphism is reversed in the closely-related black ghosts (*Apteronotus albifrons*). This reversal is accompanied by a corresponding change in endocrine regulation, whereby androgens masculinize behavior by raising EODf in brown ghosts, but by lowering 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 (*A. albifrons*) to investigate the extent of population-level variation in sexual dimorphism. We used hormone treatments to

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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 ± SD body mass; 72.1 ± 17.7 g), female n = 15 (55.7 ± 11.7 g)) and the Colombian Orinoco (abbreviated as CO; male n = 6 (67.0 ± 8.7 g), female n = 5 (48.4 ± 6.2 g)) 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 μS/cm), 25.5–27.5 °C, and 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) by using a pair of wires placed next to the fish and amplifying the signal (gain 100×; 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 min before recordings.

Playback 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 1.6 mV/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: −150 Hz, −20 Hz, −5 Hz, +20 Hz, and +150 Hz, 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 100–1000×; 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 (Kolodziejski 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 6 ms 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 3 Hz or greater over the baseline persisted for at least 10 ms but less than 30 s, and had a minimum inter-EODm interval of 100 ms. The beginning and end of each detected EODm were defined by the EODf coming within 1 Hz 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× (duration) + 10 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 (> 500 μS/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/15 mL) was added to the hormone and control solutions for visualization during earthworm preparation. Earthworms were fed to the fish every 12 h, at a dose adjusted for each fish's body mass (0.06 μg 11-kT/1 g 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 and 5 h after feeding.

Hormone assay

Blood samples were taken to verify plasma levels of gonadal steroids, 11-ketotestosterone (11-kT) and estradiol (E2). Five to 6 h after the final feeding of each treatment, blood was drawn from the hemal arch and centrifuged for 5 min 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 5 pg/mL (11-kT) and 129 pg/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 variations were 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 repeated measures analysis 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

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