Electrifying love: electric fish use species-specific discharge for mate recognition
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Mate choice is mediated by a range of sensory cues, and assortative mating based on these cues can drive reproductive isolation among diverging populations. A specific feature of mormyrid fish, the electric organ discharge (EOD), is used for electrolocation and intraspecific communication. We hypothesized that the EOD also facilitates assortative mating and ultimately promotes prezygotic reproductive isolation in African weakly electric fishes. Our behavioural experiments using live males as well as EOD playback demonstrated that female mate recognition is influenced by EOD signals and that females are attracted to EOD characteristics of conspecific males. The dual function of the EOD for both foraging and social communication (including mate recognition leading to assortative mating) underlines the importance of electric signal differentiation for the divergence of African weakly electric fishes. Thus, the EOD provides an intriguing mechanism promoting trophic divergence and reproductive isolation between two closely related Campylomormyrus species occurring in sympatry in the lower Congo rapids.

Keywords: assortative mating; Campylomormyrus; electric organ discharge; ecological speciation; weakly electric fish (mormyridae)

1. INTRODUCTION
A variety of different sensory cues are known to play a key role in mate choice (Jiggins et al. 2001; Kingston & Rossiter 2004; Bridle et al. 2006; Boul et al. 2007). These cues can drive reproductive isolation between diverging populations by facilitating assortative mating. Here, we report a case of female mate recognition (assortment) based on species-specific electrical signals in weakly electric fishes (genus Campylomormyrus) from...
containing the female had a width of 96 cm. Two zones of 32 cm each (adjacent to each male) were considered the 'preference zones'. A plastic tube was provided in the centre of each zone for shelter. Females were videotaped overnight (i.e. in darkness) under infrared illumination and the fish's location was scored every 2 min. On the second day, the experiment was repeated with males switched between sides. The total number of observations was 660 per female. To test if the EOD alone was sufficient to trigger female mate recognition, male-specific EODs were played back into the tank. Each female \(n=7\) was simultaneously presented with two digitally synthesized signals consisting of independently controlled EOD waveforms. One sequence of pulsed intervals was chosen at random from a single \(C. compressirostris\) male recorded (see the electronic supplementary material for a detailed description of the setup). The time the focal female spent in the preference zone near the active EOD playback was recorded for 1040 s (eight intervals of 130 s with signals interchanged between sides). Female preference was calculated as the proportion of observations or time spent associating with the conspecific male or playback. Data were analysed with a generalized linear mixed model of the binomial family with 'female identity' included as a random factor using R (http://www.r-project.org/).

3. RESULTS
Phylogenetic relationships and species assignment were revealed by genotyping and morphological inspection (figure 1a). In our behavioural experiment, we contrasted closely related sympatric species, \(C. compressirostris\) and \(C. rhynchophorus\), with a distant relative \(C. tamandua\). The sister species \(C. compressirostris\) and \(C. rhynchophorus\) produced very distinct EODs that differ largely in their duration. The EOD of \(C. tamandua\) was more similar to that of \(C. compressirostris\) than to \(C. rhynchophorus\) (figure 1b; table 1). In agreement with previous studies, our data did not suggest any difference between female and male EOD in \(C. compressirostris\) \((C. numenius\) morph \(C.\) (Feulner et al. 2006) revised as \(C. compressirostris\) (Feulner et al. 2007)). This was evidenced by a single species-specific cluster of data points in the PCA analysis of 10 EOD characteristics. This analysis clearly separated the three different species, but did not indicate differences between sexes within species, as both sexes were represented in our sampling.

In both experiments, \(C. compressirostris\) females preferred conspecific males over \(C. rhynchophorus\) males. By contrast, \(C. compressirostris\) females neither
discriminated between conspecific and *C. tamandua* males nor their playback signals (figure 2). Association with the conspecific male/EOD playback signal was greater when the female was given a choice between conspecific and *C. rhynchophorus* males than when the female could choose between conspecific and *C. tamandua* males (generalized linear mixed model, male stimulus: $t = -3.33$, $p = 0.007$; EOD playback: $z = -3.81$, $p < 0.001$).

### 4. DISCUSSION

Our results demonstrated that female mate recognition was influenced by EOD signals and that females associated with EODs characteristic of conspecific males. Our playback experiment was designed to evaluate the importance of the EOD waveform for species discrimination. Other possible discrimination mechanisms such as amplitude or sequence of pulse intervals were kept constant. In this way, we provided strong evidence for mate recognition between sympatrically occurring sister species based on EOD waveform characteristics.

Hence the EOD has the potential to generate assortative mating and function as a prezygotic isolation mechanism in these weakly electric fishes. Although this mechanism allowed discrimination of the closely related *C. rhynchophorus*, which showed a divergent EOD (figure 1a, b), discrimination apparently failed between EODs that are more similar to those of conspecific males (as in *C. tamandua*). Male mate recognition (Arnegard et al. 2006) and species recognition (Markowski et al. 2008) in other mormyrid genera showed an asymmetric response as well. Note here that our experiments were carried out with untrained ‘naive’ females, as trained fish do have the ability to distinguish differences smaller than intraspecific waveform variability (Paintner & Kramer 2003).

The evolution of a divergent longer EOD in *C. rhynchophorus* in conjunction with assortative mating might have promoted reproductive isolation and
dissimilarity between the closely related and sympatri-cally occurring C. rhynchosporus and C. compressirostris. This EOD divergence coincides with differences in the shape of the rostrum (figure 1a), which suggests a possible role for trophic niche segregation (Feulner et al. 2007). The EOD might be a trait allowing direct transmission of ecologically caused divergent selection to a form of reproductive isolation (Kirkpatrick & Ravigne 2002; Rundle & Nosil 2005). Indeed, there is evidence that the EOD is involved in foraging for insect larvae in electric fish (von der Emde & Bleckmann 1998) and EOD duration might be adaptive for the detection of different preys (Meyer 1982; von der Emde & Ringer 1992). The dual function; (i) electrolocation used in foraging and (ii) social communication, including assortative preferences (as evidenced in this study), points towards ecological divergence pleiotropically effecting assortative mating in this system.

We argue that species-specific changes in EOD linked to female preference for that signal, provide an intriguing mechanism for divergence through assortative mating. The electric sense can be considered a key innovation not only regarding its primary function, i.e. electrolocation, but also as an efficient trait for ‘electric’ mate recognition.

Experiments were approved by the Deputy for Animal Welfare at the University of Potsdam to comply with legal requirements.

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