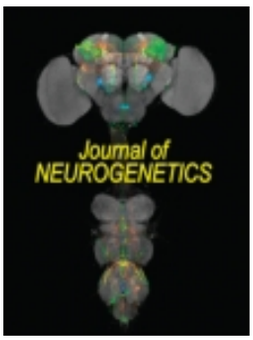

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Review

From Sequence to Spike to Spark: Evo-devo-neuroethology of Electric Communication in Mormyrid Fishes

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Abstract: Mormyrid fishes communicate using pulses of electricity, conveying information about their identity, behavioral state, and location. They have long been used as neuroethological model systems because they are uniquely suited to identifying cellular mechanisms for behavior. They are also remarkably diverse, and they have recently emerged as a model system for studying how communication systems may influence the process of speciation. These two lines of inquiry have now converged, generating insights into the neural basis of evolutionary change in behavior, as well as the influence of sensory and motor systems on behavioral diversification and speciation. Here, we review the mechanisms of electric signal generation, reception, and analysis and relate these to our current understanding of the evolution and development of electromotor and electrosensory systems. We highlight the enormous potential of mormyrids for studying evolutionary developmental mechanisms of behavioral diversification, and make the case for developing genomic and transcriptomic resources. A complete mormyrid genome sequence would enable studies that extend our understanding of mormyrid behavior to the molecular level by linking morphological and physiological mechanisms to their genetic basis. Applied in a comparative framework, genomic resources would facilitate analysis of evolutionary processes underlying mormyrid diversification, reveal the genetic basis of species differences in behavior, and illuminate the origins of a novel vertebrate sensory and motor system. Genomic approaches to studying the evo-devo-neuroethology of mormyrid communication represent a deeply integrative approach to understanding the evolution, function, development, and mechanisms of behavior.

Keywords: electric organ, electrocommunication, electrocyte, electroreception, evolution, motor control, speciation, sensory processing

A MODEL SYSTEM FOR UNDERSTANDING EVOLUTIONARY DEVELOPMENTAL MECHANISMS OF BEHAVIORAL DIVERSIFICATION

Evolutionary developmental biology (evo-devo) seeks to understand how evolutionary change in developmental processes determines the diversity of life on earth (Carroll, 2000, 2008; Hall, 2000; Rudel & Sommer, 2003; Sommer, 2009). The field is representative of a larger movement to integrate proximate and ultimate explanations in biology (see Mayr, 1961) by studying mechanisms in a comparative and functional context (Carlson, 2012; MacDougall-Shackleton, 2011; McNamara & Houston, 2009; Ryan, 2005; Sherry, 2005, 2006). This attempt at integration has often resulted in confusion and controversy (Alcock & Sherman, 1994; Dewsbury, 1994; Francis, 1990; Mayr, 1993; Sherman, 1988; Thierry, 2005), but it has also led to novel insights when a clear distinction between proximate and ultimate causes is maintained. Evo-devo holds the promise of revealing how phenotypic variation and evolutionary novelty are realized through the identification of genetic mechanisms underlying development (Wagner & Lynch, 2010).

One of the core principles emerging from evo-devo is that morphological differences among species result primarily from evolutionary changes in the regulation of gene expression during development rather than changes in protein coding sequences (Carroll, 2000, 2008). In particular, mutations in *cis*-regulatory elements are thought to be the main driver of evolutionary change, determining which genes are expressed, where they are expressed in the body, and at what times during development (Carroll, 2008; Wagner & Lynch, 2010; Wray, 2007). The highly

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modular nature of *cis*-regulatory elements, each one independently regulating the expression of a particular transcript, means that mutations to *cis*-regulatory regions can alter gene expression patterns while avoiding negative pleiotropic effects and the disruption of developmental networks (Carroll, 2008). However, some have criticized the strong emphasis on *cis*-regulatory elements being the main drivers of evolutionary change as premature and potentially inaccurate (Hoekstra & Coyne, 2007). In particular, gene duplication followed by divergence appears to be a common route by which changes to protein coding regions can lead to evolutionary novelty and adaptation while avoiding the negative consequences of pleiotropy (Arnegard et al., 2010b; Dehal & Boore, 2005; Ohno, 1970; Taylor & Raes, 2004; Zhang et al., 2002). In addition, comparative studies point to rapid evolution of protein coding sequences in primates (Bustamante et al., 2005; Clark et al., 2003; Dorus et al., 2004; Nielsen et al., 2005).

Evo-devo has largely focused on the genetic developmental mechanisms of morphology (Carroll, 2008; Hoekstra & Coyne, 2007). There are several reasons for this: morphology is readily observable on a variety of spatial and temporal scales, within and between organisms; it is often relatively straightforward to deduce the functional significance of morphological features; and morphology is typically the only aspect of phenotype preserved in the fossil record. However, it is not clear whether insights related to the genetic basis of morphological development can generalize to evolutionary change in physiology, biochemistry, and behavior (Hoekstra & Coyne, 2007). In contrast to the evo-devo emphasis on *cis*-regulatory elements, there are several examples of adaptive divergence in physiology resulting from changes in coding sequences (Cheng, 1998; Duman, 2001; Fletcher et al., 2001; Hughes, 2002; Jessen et al., 1991; Yokoyama, 2002; Zakon et al., 2006; Zhang, 2006). It is possible, though by no means certain, that the evolution of morphological and nonmorphological traits result from fundamentally different genetic mechanisms. Regardless, evolutionary biology as a discipline is interested in understanding the general processes of adaptation and speciation as they relate to all aspects of phenotype (Losos et al., 2013).

Like morphology, behavior is a defining characteristic of species that is remarkably diverse and shaped by both genetics and environmental plasticity. Behavior can also establish reproductive isolation, and therefore drive speciation and the emergence of biodiversity (Hoskin & Higgie, 2010; Panhuis et al., 2001; Ptacek, 2000; Ritchie, 2007; West-Eberhard, 1983). Compared with morphology, however, behavior is typically complex and context dependent, making it more difficult to determine its underlying mechanisms. Nevertheless, neuroethologists have made great progress in revealing neural mechanisms of behavior by focusing on natural behaviors in model systems that are particularly accessible, amenable to study, or highly specialized for these behaviors (Hoyle, 1984; Katz, 2010; Pflüger & Menzel, 1999). One promising avenue for studying the genetic basis of behavioral diversification and speciation is to pursue an evo-devo approach, but apply it to a group of organisms that are phenotypically diverse and that fit the neuroethological model of being well-suited to establishing links between multiple levels of inquiry such as genetics, morphology, physiology, and behavior. "Evo-devo-neuroethology" represents a deeply integrative approach that seeks to determine the ultimate and proximate causes and consequences of behavioral diversity.

Weakly electric fish have long been a model system of choice for neuroethologists (Heiligenberg, 1991; Moller, 1995; Rose, 2004; Sawtell et al., 2005; Zakon, 2003). African fishes in the family Mormyridae generate pulses of electricity for the purposes of communication and active sensing (Hopkins, 1986a). Their electric signals are simple and short, making them easy to record, quantify, manipulate, and reproduce. At the same time, their electric signals are remarkably diverse and can be used to communicate a rich variety of context-dependent social signals (Carlson, 2002a). We have a thorough understanding of the neural basis of signal production and variation, so that recording electric signals in freely behaving fish provides a direct window into their electromotor system (Bass, 1986a; Caputi et al., 2005; Carlson, 2002a; Hopkins, 1999). The sensory processing of electric signals occurs in a dedicated sensory pathway that allows for the successful integration of in vivo studies of information processing with in vitro studies of cellular, synaptic, and circuit mechanisms (Amagai, 1998; Amagai et al., 1998; Carlson, 2009; Carlson et al., 2011; Friedman & Hopkins, 1998; George et al., 2011; Lyons-Warren et al., 2012; Lyons-Warren et al., in review; Ma et al., in review). Thus, we are beginning to understand the neural basis of sensory perception in the context of communication behavior at an unprecedented level of detail (Baker et al., in press).

More recently, weakly electric fish have also emerged as a model system for studying the role of communication systems in species diversification. Mormyrids are speciose ($>$200 described species) and characterized by a high degree of phylogenetic and phenotypic diversity (Arnegard et al., 2010a; Eschmeyer & Fricke, 2011; Feulner et al., 2007; Hopkins et al., 2007; Lavoué et al., 2003, 2008, 2010; Sullivan et al., 2000). Electric signaling represents a fairly open channel of communication, relatively free from constraints imposed by predators, competing signals, background noise, and environmental effects on signal transmission (Arnegard et al., 2010a; Hopkins, 1986b, 1999). Several lines of evidence point to electric communication as a major driver of mormyrid diversification (Arnegard et al., 2010a; Carlson & Arnegard, 2011; Carlson et al., 2011; Feulner et al., 2009b; Lavoué et al., 2008).

It is clear that mormyrids are an excellent model system for studying the evolution of neural mechanisms responsible for diverse behaviors. The one remaining limitation to fully developing this system is the current lack of genomic and transcriptomic resources. Our purpose here is to highlight the unique advantages of mormyrid fishes as a model system for understanding evolutionary developmental mechanisms of behavioral adaptation and diversification, and to make the case for developing genomic resources in mormyrids so that we may realize their full potential.

WHO? WHAT? WHERE? ELECTRIC COMMUNICATION IN MORMYRID FISHES

Mormyrids communicate using pulses of electricity termed electric organ discharges, or EODs (Hopkins, 1981). The EOD waveform is species specific (Figure 1A). In many species, the EOD waveform also differs between the sexes (Carlson & Arnegard, 2011; Hopkins, 1986a; Lavoué et al., 2008). In most cases, these sex differences arise at the onset of the breeding season (Bass & Hopkins, 1983, 1985; Bass & Volman, 1987). Breeding males typically have elongated EODs compared with females, and in some cases they have a distinct waveform. Playback experiments in a small handful of species have revealed that these species and sex differences in EOD waveform are used for species recognition and mate choice (Arnegard et al., 2006; Feulner et al., 2009a; Hopkins & Bass, 1981; Machnik & Kramer, 2008). However, a recent comparative study suggests that this is not true for all lineages: most species within the subfamily Mormyrinae can detect variation in EOD waveform, whereas most species in the other mormyrid subfamily, Petrocephalinae, cannot (Carlson et al., 2011). This perceptual difference is associated with much greater EOD waveform diversity among the Mormyrinae (Figure 1A).

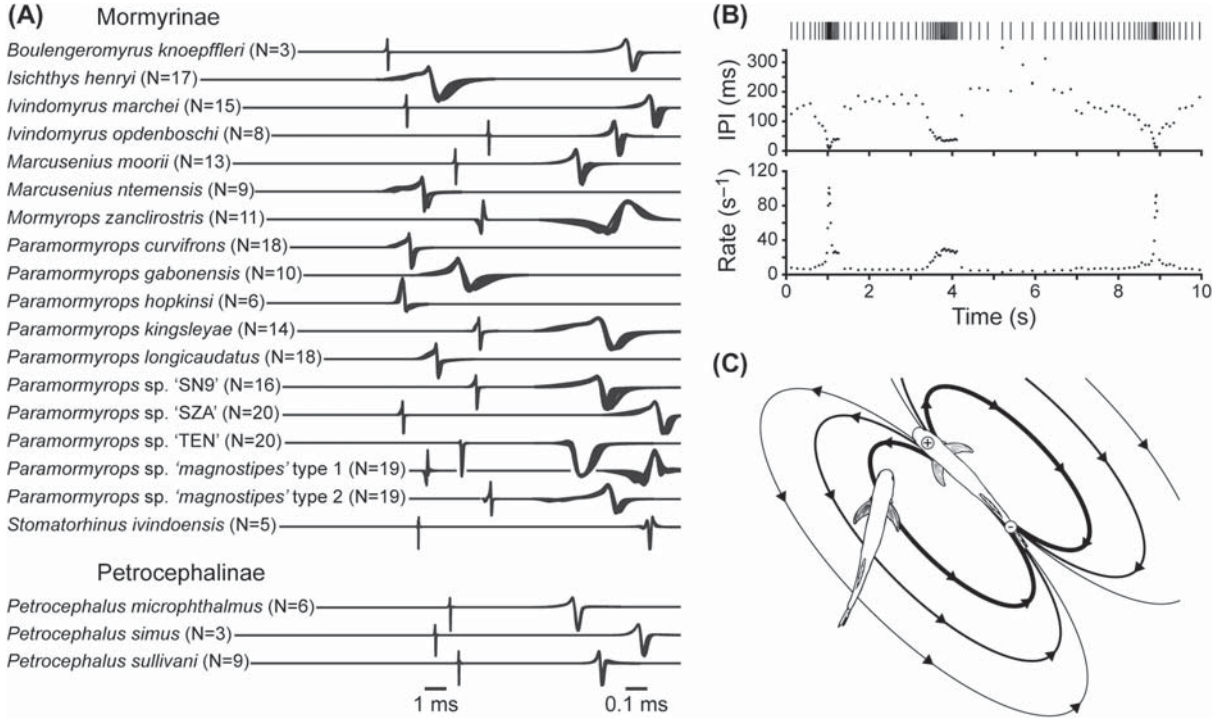

Figure 1. Mormyrid electric signals communicate information about identity (*who?*), behavioral state (*what?*), and location (*where?*). (**A**) Electric organ discharge (EOD) waveforms recorded from 21 species from the Ivindo River of Gabon plotted on two different timescales (modified from Carlson et al., 2011). There are two different subfamilies of mormyrids, the Mormyrinae and the Petrocephalinae, of which there are 18 and 3 known species within the Ivindo River, respectively. EOD waveforms from different individuals are normalized to the same peak-to-peak height, superimposed, and aligned to the head-positive peak (except for *Paramormyrops* sp. "TEN," for which waveforms are aligned to the head-negative peak). The EOD waveform is species specific and in many species there are also sex and individual differences in EOD waveform. (**B**) Sequence of interpulse intervals (IPIs) recorded from a freely behaving *Brienomyrus brachyistius* (modified from Carlson and Hopkins, 2004b). The train of EOD pulses is shown at the top (each vertical tick represents one EOD), and these are represented as both IPIs and instantaneous rates below. Mormyrids actively vary IPIs to communicate behavioral state. (**C**) During electric communication, the current flow through the body of the receiving fish depends on the relative location and orientation of the signaling fish. Here, the signaling fish is represented as an electric dipole, causing current to flow from head to tail (represented by arrows) and to attenuate with distance (represented by line thickness). As a result, the receiving fish receives inward current on its left side (positive polarity) and outward current on its right side (negative polarity), as well as a stronger current at the head compared with the tail.

Individual differences in EOD waveform have also been described (Friedman & Hopkins, 1996). At least some of this variation relates to social hierarchy (Carlson et al., 2000; Terleph & Moller, 2003). In *Brienomyrus brachyistius*, dominant breeding males have longer EODs than subordinates (Carlson et al., 2000). Individual variation in the EOD raises the possibility that it may be used for individual recognition. Although a formal test of this has not yet been performed, a conditioning paradigm suggests that mormyrids are able to detect individual differences in EOD waveform (Paintner & Kramer, 2003).

The timing of each EOD is highly variable, with interpulse intervals (IPIs) ranging from ~10 ms to as long as several seconds (Figure 1B). Mormyrids actively vary IPIs during social interactions, and distinct sequences of IPIs have been linked to a variety of behavioral contexts (reviewed in Carlson, 2002a), including courtship and mating (Bratton & Kramer, 1989; Wong & Hopkins, 2007), aggression and territoriality (Bell et al., 1974; Kramer & Bauer, 1976), schooling (Moller, 1976), and pack hunting (Arnegard & Carlson, 2005). There are few playback studies that have assessed behavioral responses to IPIs. However, they do suggest that IPIs recorded from fish in different behavioral contexts influence the behavior of receivers in different ways (Kramer, 1979), and that these responses depend on the natural sequence of IPIs (Teyssedre & Serrier, 1986). Few comparative studies of IPIs have been performed, but descriptions of electric signaling in different species suggest differences in IPI distributions as well as specific IPI patterns (reviewed in Carlson, 2002a). There is evidence that some species may use IPIs for species recognition (Carlson & Arnegard, 2011; Kramer & Kuhn, 1994).

In addition to determining who the signaling fish is and what they are communicating, mormyrids also use electric signals to determine where the signaling fish is located (Schluger & Hopkins, 1987). The electric organ behaves like a dipole, generating an electric field that surrounds the fish (Hopkins, 1986b). The direction and intensity of the resulting electric field varies in space, causing the transepidermal current to vary across the body surface (Figure 1C). Receiving fish locate signaling fish by orienting to these electric current lines and following them to the source (Schluger & Hopkins, 1987).

FROM SPIKES TO SPARKS: THE NEURAL BASIS OF ELECTRIC SIGNALING

Central Control of Electric Signaling

EOD production is controlled by an electromotor network in the hindbrain medulla that determines EOD timing, and therefore the sequence of IPIs (Figure 2). Each EOD is initiated in a midline command nucleus, which drives

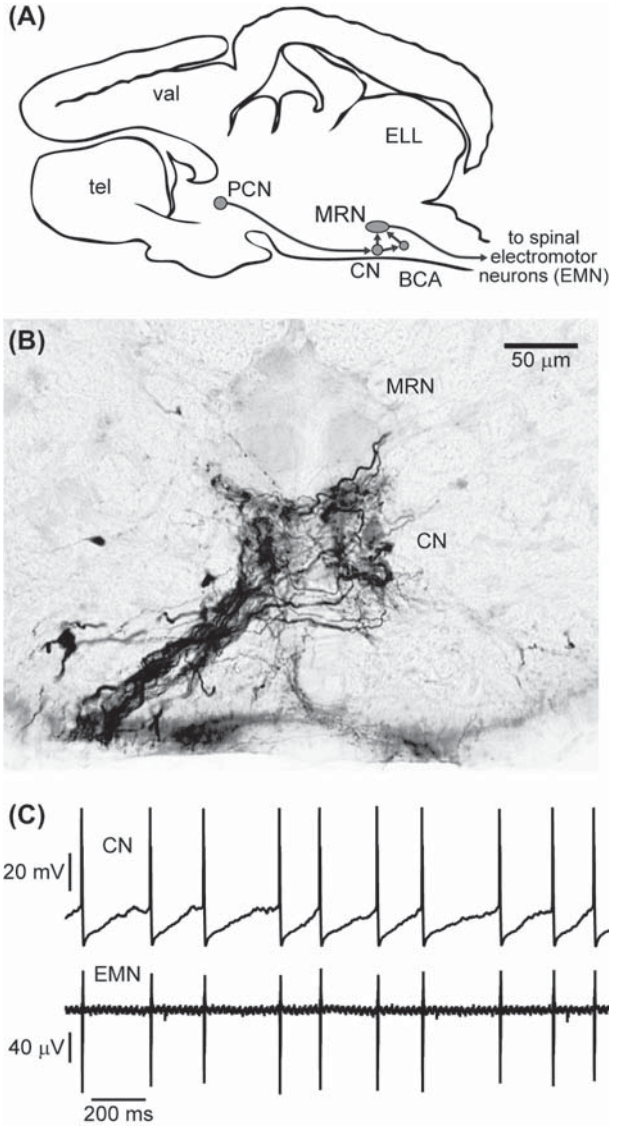

Figure 2. The timing of EOD production is controlled by a central electromotor pathway. (**A**) A sagittal schematic of the mormyrid brain (modified from Carlson, 2002a). The medullary command nucleus (CN) determines the timing of each EOD. It integrates descending excitatory input from precommand nuclei (PCN) in the midbrain/diencephalon, and sends its output to the medullary relay nucleus (MRN) and the bulbar command-associated nucleus (BCA), which provides a second, indirect input to the MRN. The axons of MRN neurons project down the spinal cord to innervate spinal electromotor neurons (EMN) that directly control the electric organ. (**B**) A transverse section through the medulla of *B. brachyistius* (modified from Carlson, 2002b). Descending fibers from the PCN are labeled with neurobiotin, and they can be seen terminating in the midline CN. Large MRN neurons are visible just dorsal to CN. (**C**) A simultaneous intracellular recording from a CN neuron and extracellular recording from EMN reveals the 1:1 relationship between CN spiking and EOD output. In this recording, the fish is paralyzed and not generating an EOD, but EMN activity represents a fictive EOD. Gradual depolarization of the CN neuron's membrane potential leading up to each spike reflects the integration of ongoing excitatory input from PCN (Carlson, 2003).

spinal electromotor neurons to spike in synchrony via an indirect projection through medullary relay neurons (Bell et al., 1983; Bennett et al., 1967; Grant et al., 1986). Extensive electrical coupling helps ensure the synchronous activation of electrocytes, the excitable cells in the electric organ that actually generate the EOD (Bennett et al., 1963, 1967; Elekes et al., 1985; Elekes & Szabo, 1985; Grant et al., 1986).

The command nucleus also gives rise to an ascending corollary discharge pathway that provides motor-related inputs to electrosensory regions (Bell et al., 1983; Carlson, 2002b). These corollary discharge inputs allow the fish to distinguish self-generated from external electrosensory stimuli, which is important for active electrolocation and communication, respectively (Bell, 1989). They also allow for the adaptive filtering of electrosensory information as environmental conditions change during active electrolocation (Bell, 2001).

The output of the command nucleus is primarily influenced by descending excitatory inputs from the midbrain precommand nucleus and the dorsal posterior thalamic nucleus (Bell et al., 1983; Carlson, 2002b, 2003; von der Emde et al., 2000). These two nuclei are likely responsible for the production of different communication signals (Carlson & Hopkins, 2004a). Both descending nuclei receive inhibition from the corollary discharge pathway, which silences their activity just after the production of each EOD (Carlson, 2003; von der Emde et al., 2000). This recurrent inhibition appears to regulate EOD output and may be involved in the production of different displays (Carlson & Hopkins, 2004a).

The central electromotor pathway has been studied in detail in just two species, *B. brachyistius* and *G. petersii*. Thus, we don't yet know the extent of evolutionary change in the central electromotor pathways of mormyrids. The central electromotor pathways of the distantly related South American gymnotiforms have been better studied, revealing species differences in anatomical wiring and neurochemistry that underlie species differences in electric signaling behavior (reviewed in Kawasaki, 2011). Unfortunately, we know very little about the development of central electromotor pathways.

Morphophysiological Basis of Electric Signal Variation

Mormyrid EOD waveforms vary in duration, polarity, number of phases, and rates of voltage change over time (Figure 1A). The morphological and electrophysiological properties of electrocytes in the electric organ determine these properties of the EOD (reviewed in Bass, 1986a; Bennett, 1971; Caputi et al., 2005). The mormyrid electric organ is composed of four axially oriented columns, each consisting of approximately 20–100 disc-shaped electrocytes (Figure 3). Mormyrid electrocytes all have a protruding stalk system that originates from one face of the electrocyte and that receives innervation from spinal electromotor neurons (Hopkins, 1999). These stalks may reverse course and penetrate the electrocyte membrane once or twice, leading to a remarkable diversity of electrocyte morphologies, including nonpenetrating with posterior innervation (NPp), penetrating with anterior or posterior innervation (Pa or Pp, respectively), doubly penetrating with posterior innervation (DPp), or doubly penetrating and nonpenetrating with posterior innervation (DPNP).

Both the anterior and posterior faces of mormyrid electrocytes are electrically excitable (Bennett, 1971). The electrocytes are almost always morphologically identical within a fish and they depolarize synchronously such that the EOD waveform is a sum of the micro-EODs recorded from each individual electrocyte (Bennett, 1971; Bennett & Grundfest, 1961). Electrocyte morphology and innervation governs current flow within the electrocyte, thereby determining the polarity and number of phases in the EOD waveform (Figure 4). In electrocytes with NPp anatomy, depolarization of the stalk followed by the posterior face result in current flow towards the head, generating the initial head positive phase, P1. This flow of current causes the depolarization of the anterior face after a short delay, causing current to flow in the opposite direction, generating a late head negative phase, P2. Electrocytes with Pa anatomy are innervated on the anterior side, causing the stalk current to flow towards the tail before activating the posterior face, resulting in a relatively small head negative phase, P0, that precedes the head-positive P1 (Bass, 1986a; Bennett, 1971; Gallant et al., 2011). As with NPp-type electrocytes, the current from P1 activates the anterior face, generating a head-negative P2. Species with Pp electrocyte anatomy also have a triphasic EOD, but the head-tail polarity is reversed. Electrocytes may also vary in the total number of stalk penetrations, which is correlated with the magnitude of the early P0 phase (Bennett & Grundfest, 1961; Gallant et al., 2011). Species differences in EOD duration may be related to the number of surface invaginations in the anterior face of the electrocyte, which contributes to surface area and may influence passive electrical properties (Bass et al., 1986).

There are little data available on the ionic basis of EODs in mormyrids. This partly results from considerable difficulties in recording from mormyrid electrocytes, which are ensheathed in tough connective tissue. We know much more about the ionic basis of EODs in gymnotiforms, which result from inward Na^+ currents and delayed rectifying outward K^+ currents (Dunlap et al., 1997; Ferrari et al., 1995; Ferrari & Zakon, 1993; Markham & Stoddard, 2005; Markham et al., 2009; McAnelly et al., 2003; McAnelly & Zakon, 1996, 2000; Mills & Zakon, 1987; Zakon et al., 1999).

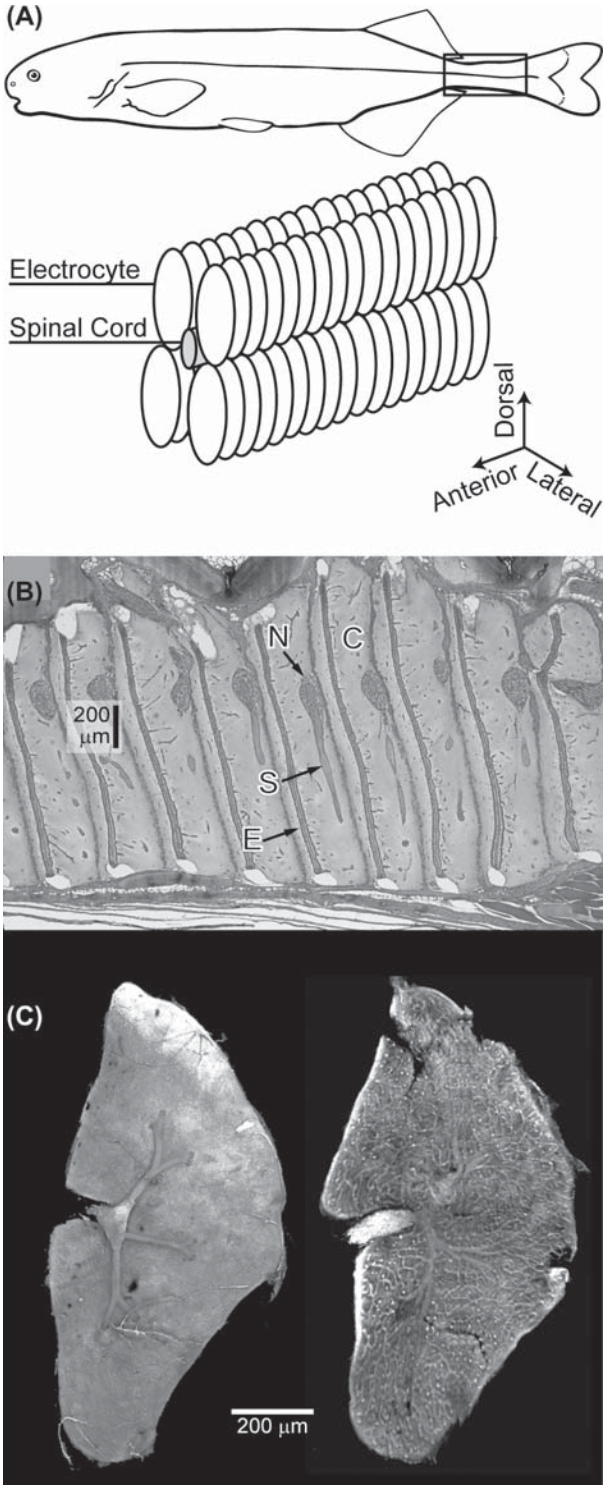

Figure 3. Electric organ anatomy. (**A**) Electric organs are located in the caudal peduncle, denoted by the boxed area, and are composed of four columns of electrocytes surrounding the spinal cord, ensheathed in tough connective tissue. (**B**) A saggital section through the electric organ of *Paramormyrops hopkinsi* shows individual electrocytes (E) bounded by connective tissue septa (C). The electrocytes of *P. hopkinsi* are nonpenetrating (NPp). Thus, the stalk (S) corresponding to each electrocyte is located posterior to the electrocyte. Small stalklets that branch off of each stalk can be seen emanating from the posterior face of each electrocyte. The electromotor nerves (N) that innervate the stalk system can be seen contacting stalks. (**C**) Confocal micrographs of two electrocytes from *P. kingsleyae*. The image on the left is of a Pa-type electrocyte viewed from the anterior side, where eight penetrations of the stalk are visible. The image on the right is of a NPp-type electrocyte viewed from the posterior side, where all of the stalk system can be seen without any penetrations.

Hormonal Control of Sex Differences in Electric Signaling

Studies of natural and laboratory populations of mormyrids have revealed that sex differences in EOD waveform are caused by increased levels of gonadal steroid hormones at the onset of the breeding season (Bass, 1986b). When females, juveniles, or nonreproductive males are treated with testosterone or dihydrotestosterone (DHT), EOD waveforms elongate over a period of 2–3 weeks (Bass & Hopkins, 1983). Estradiol also affects the EOD, but to a much lesser extent than androgens (Bass & Hopkins, 1983). In breeding populations of *B. brachyistius*, status-dependent differences in the EOD durations of males are correlated with circulating levels of plasma 11-ketotestosterone (Carlson et al., 2000).

The androgen sensitivity of electric organ is mediated by a 4- to 5-fold greater number of androgen receptors compared with trunk muscle (Bass, 1986a). Androgen-induced changes in EOD waveform are associated with structural changes in the electric organ (Bass et al., 1986). Over the course of androgen treatment, there is an increase in electrocyte thickness due to an increase in the thickness and number of surface invaginations of the anterior face. These morphological changes are linked to a concomitant increase in the duration of electrocyte action potentials (Bass & Volman, 1987). It is presently unknown how androgens mediate these structural changes to electric organs.

Evolution and Development of Electric Organs

We presently know of six independent origins of electric organs in fishes: torpedinoids, rajoids, mormyroids, gymnotiforms, siluriforms, and uranoscopids (Bass, 1986a). In all but one family of gymnotiforms, the Apteronotidae, electric organs are derived during development from skeletal muscle tissue (Bass, 1986a; Bennett, 1971). Both mormyroids and gymnotiforms evolved electric organs that originate developmentally from myogenic precursors (Patterson & Zakon, 1997), and the transition between skeletal muscle and electric organ is accompanied by a substantial change in cell size (Unguez & Zakon, 1998a, 1998b), morphology (Denizot et al., 1982), and physiology

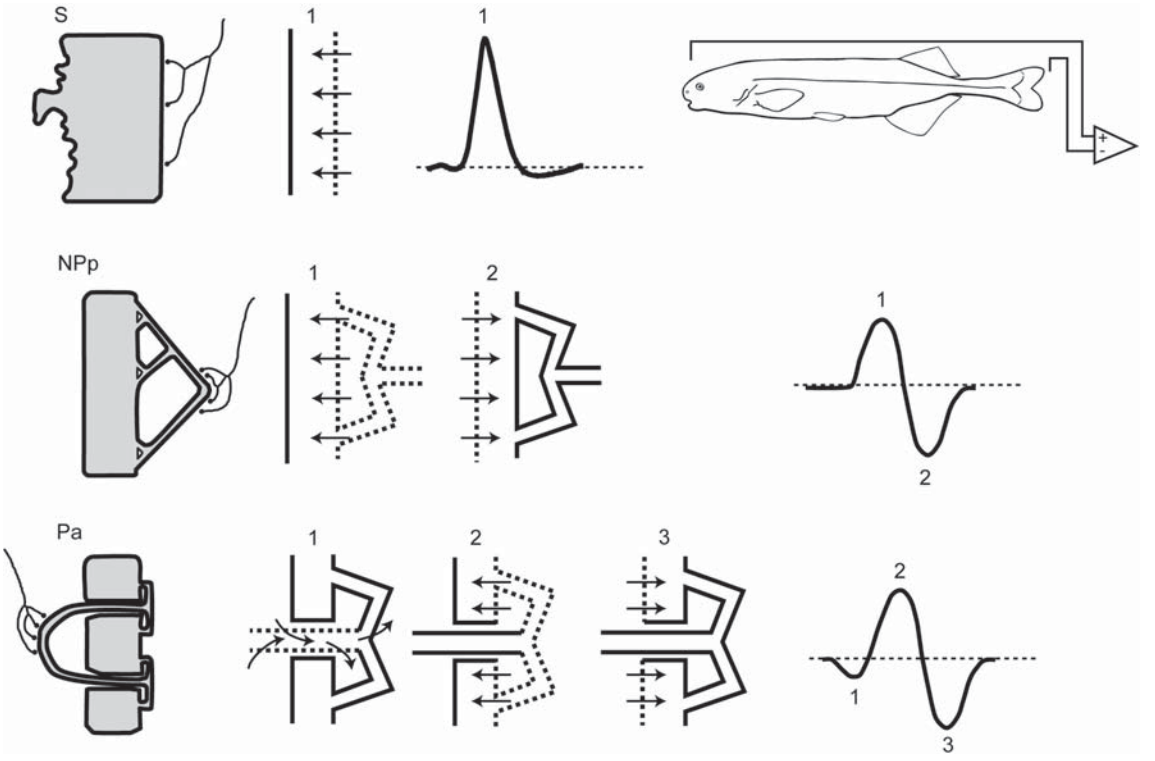

Figure 4. Electrocyte anatomy and the stalk system govern current flow through electrocytes, determining EOD polarity and number of phases. Schematics of different electrocyte anatomies are shown, and the sequence of membrane excitation and current flow during each EOD is indicated by dashed lines and arrows, respectively. On the far right, the EOD waveform recorded between the head and tail is indicated. Stalkless electrocytes (S) are found only in *Gymnarchus niloticus*, the sister taxon to all mormyrids. They produce monophasic EODs resulting from direct innervation of the excitable posterior electrocyte face by electromotor neurons, causing current to flow towards the head. Nonpenetrating with posterior innervation (NPp) electrocytes have two excitable faces and produce biphasic EODs. The stalk system is innervated on the posterior side by electromotor neurons, and the resulting depolarization propagates to the posterior face. Depolarization of the stalk and posterior face causes current to flow towards the head, resulting in a "head-positive" phase (1). This current flow results in delayed excitation of the anterior face, which causes current to flow towards the tail, resulting in a "head-negative" phase (2). Penetrating with anterior innervation (Pa) electrocytes produce triphasic EODs. The stalk is innervated by electromotor neurons on the anterior side, and then passes through the electrocyte to the posterior side. The flow of current through the stalk leads to current flow towards the tail, resulting in a head negative phase (1), followed by excitation of the posterior face, leading to current flow towards the head (2), and finally excitation of the anterior face causing current to flow towards the tail (3).

(Westby & Kirschbaum, 1977), which ultimately leads to the retention of electrical excitability without the generation of contractile force. Interestingly, mormyrids first develop a functional larval electric organ about 8 days after fertilization, and then later develop a separate adult electric organ (Denizot et al., 1978). Both electric organs have their own populations of spinal electromotor neurons, but both are controlled by the hindbrain command nucleus (Kirschbaum, 1981; Kirschbaum et al., 1979). Although the larval electric organ eventually degenerates, there is a period of several days over which both electric organs are functional, resulting in the production of both larval and adult EODs (Denizot et al., 1978). The larval electric organ is difficult to distinguish histologically from skeletal muscle, whereas the adult electric organ is more clearly differentiated.

What causes muscle to become electric organ? One hypothesis has been that motor neurons innervating the electric organ induce the developmental transition between skeletal muscle and electric organ (Patterson & Zakon, 1997; Szabo & Kirschbaum, 1983; Unguez & Zakon, 1998a). A recent study by Cuellar et al. (2006) has found that several sarcomeric genes are transcribed in the electric organ of gymnotiforms, but the proteins are not translated. This has motivated the hypothesis that motor neuron activity in gymnotiform electric organ may suppress skeletal-muscle-specific gene expression through posttranscriptional regulation. This seems unlikely in mormyrids, however, given that electric organs persist, even in the absence of innervation. Transcription factors involved in the early development of muscle may somehow be involved in the transition to electric organ (Kim et al., 2008). Intriguingly, many of these transcription factors are up-regulated (e.g., MyoD, myogenin, myf5, and MRF4), whereas others (e.g., MEF2c, Id1, and Id2) are not (Kim et al., 2008).

Gallant et al. (2012) compared gene expression in skeletal muscle and electric organ in mormyrids, and identified 120 genes that are differentially expressed between the two tissues. The results of this study indicate that mormyrids, unlike gymnotiforms, retain expression and translation of several sarcomeric proteins during the developmental transition from skeletal muscle to electric organ. Instead of expressing the same form in skeletal muscle and electric organ, mormyrids seem to express electric-organ-specific isoforms of these genes. In addition, Gallant (2012) demonstrated the differential expression of a critical transcription factor, myocyte enhancer factor 2A (MEF2a), which is up-regulated in the electric organ.

Evolutionary Developmental Mechanisms of Electrogenic Diversity

Histological studies have described the nature of anatomical variation in electric organs in a variety of mormyrid species (Alves-Gomes & Hopkins, 1997; Bass, 1986a; Bennett & Grundfest, 1961; Gallant et al., 2011; Hopkins, 1999; Sullivan et al., 2000). *Gymnarchus niloticus* is the monotypic sister taxon to all mormyrids (Alves-Gomes & Hopkins, 1997; Sullivan et al., 2000). It lacks a stalk system, and the structure and organization of its electric organ is completely different from the adult mormyrid electric organ (Denizot et al., 1978, 1982; Kirschbaum, 1987). The *Gymnarchus* electric organ is more similar to the larval mormyrid electric organ, suggesting that stalkless electrocytes are a shared ancestral mormyroid trait and that stalked electrocytes represent a more derived trait found in the common ancestor of all mormyrids (Sullivan et al., 2000) (Figure 5).

Within the mormyrids, the two subfamilies Petrocephalinae and Mormyrinae are sister taxa. All petrocephalines that have been studied have NPp-type electrocytes without any stalk penetrations. The electrocytes of mormyrines are far more diverse, including species having NPp, Pa, Pp, DPp, and DPNP anatomies (Figure 5). In the few mormyrine species that have been studied, electrocytes with penetrating stalk anatomies pass through an early developmental stage in which they lack penetrations (Denizot et al., 1982; Hopkins, 1999; Sullivan et al., 2000; Szabo, 1960). Thus, NPp appears to be the primitive condition for mormyrids, with penetrating stalks first arising in the common ancestor of the Mormyrinae (Figure 5). Within the Mormyrinae, there have been multiple independent reversions to non-penetrating stalks, thought to represent pedomorphic evolutionary changes in development (Alves-Gomes & Hopkins, 1997; Hopkins, 1999; Sullivan et al., 2000). Hopkins (1999) and Alves-Gomes and Hopkins (1997) describe two physical mechanisms by which penetrating stalks may arise during development, both involving the physical migration of stalk through the developing electrocyte membrane. These models suggests two potential points of "arrested development" in electrocyte ontogeny that could contribute to electrogenic diversity in the Mormyrinae, one in which there is no movement of the stalk system, causing a reversion to NPp-type electrocytes, and another in which the proximal ends of the stalks fail to penetrate the electrocyte, resulting in DPp-type electrocytes.

Recent molecular studies reveal that independent, parallel evolutionary change in a gene that codes for voltage-gated sodium channels is related to the evolution of electrogenesis in both gymnotiforms and mormyroids (Arnegard et al., 2010b; Zakon et al., 2006, 2008). The gene *scn4a* encodes the muscle-specific sodium channel *Nav*1.4 in vertebrates. A teleost-specific whole-genome duplication event ca 200–300 million years ago (MYA) resulted in two orthologs of *scn4a*, *scn4aa* and *scn4ab*, which code for the *Nav*1.4a and *Nav*1.4b channels, respectively. In all nonelectrogenic teleosts studied, both forms are expressed in skeletal muscle, but in both gymnotiforms and mormyroids *scn4aa* is expressed only in electric organ and *scn4ab* is expressed only in skeletal muscle (Arnegard et al., 2010b; Zakon et al., 2006). Both lineages have experienced strong positive selection on *scn4aa* (Arnegard et al., 2010b; Zakon et al., 2006, 2008), and in both cases the amino acid substitutions are in regions that control the activation and inactivation kinetics of *Nav*1.4a (Arnegard et al., 2010b). These mutations may contribute to EOD waveform diversity by altering the kinetics of sodium currents in electrocytes. Importantly, the compartmentalization of *scn4aa* in electric organ and *scn4ab* in muscle allowed for selection to act on *scn4aa* to diversify electric signaling without negatively affecting muscle function (Zakon et al., 2006). This striking example of parallel molecular evolution highlights the potential of gene duplication to foster evolutionary innovation.

FROM SPARKS TO SPIKES: THE NEURAL BASIS OF ELECTROSENSORY PROCESSING

Electric Signals Are Detected by Specialized Communication Sensors

Mormyrids have three distinct types of electroreceptor organs, each one specialized for a different function (reviewed in Zakon, 1986). Ampullary electroreceptors mediate detection of weak, low-frequency electric fields. They are found in both electric and several nonelectric fishes, and they function in passive electrolocation. By contrast, tuberous receptors are found only in electric

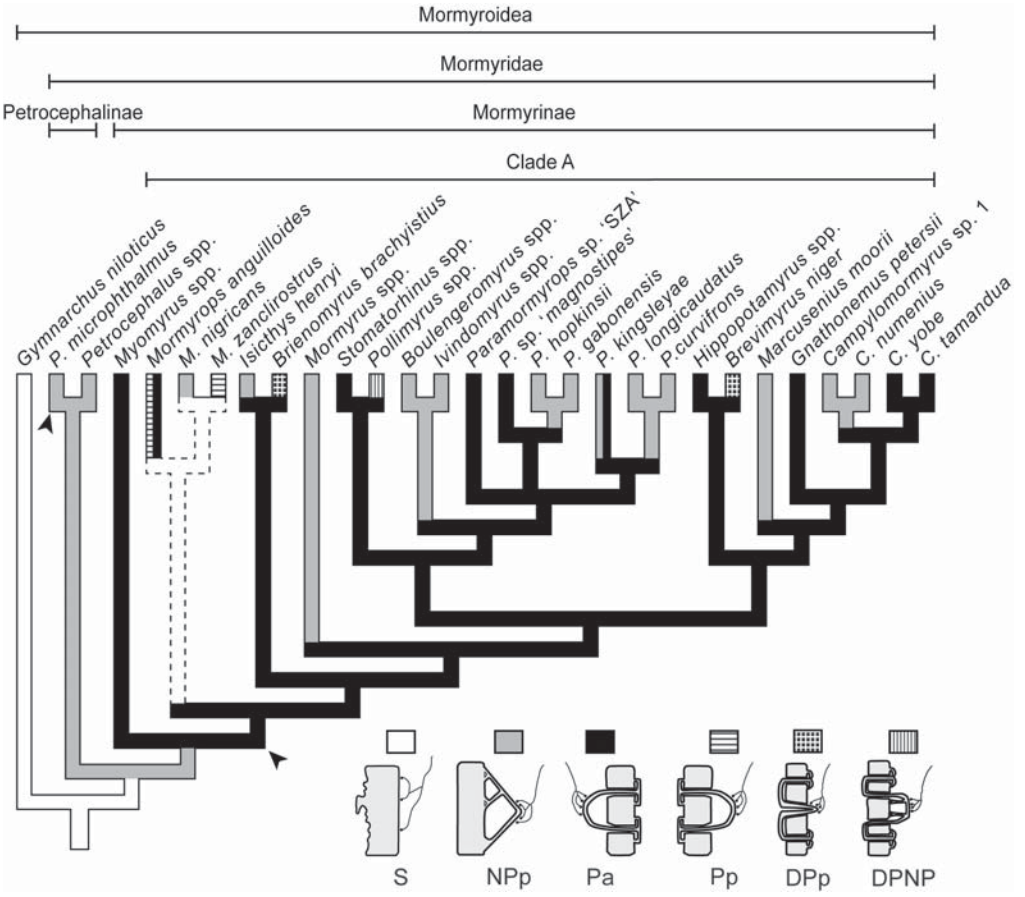

Figure 5. A cladogram representing the phylogenetic relationships of mormyrids, based on Sullivan et al (2000), Carlson et al. (2011), and Lavoué et al (2012). The phylogenetic distribution of electrocyte anatomy and the hypothesized history of electrocyte evolution is shown (see Sullivan et al., 2000, 2004). In addition, evolutionary origins for the sensory brain region ELa/ELp are indicated by arrowheads, one at the base of *P. microphthalmus* and another at the base of clade A (Carlson et al., 2011). Stalkless (S) electrocytes are thought to be the ancestral mormyroid condition, with stalks arising at the origin of mormyrids in the form of nonpenetrating with posterior innervation (NPp). Penetrating stalks with anterior innervation (Pa) arose at the origin of the subfamily Mormyrinae. Within clade A of the Mormyrinae, there appear to have been multiple reversals to NPp-type electrocytes, as well as the evolution of penetrating stalks with posterior innervation (Pp), doubly penetrating stalks with posterior innervation (DPp), and doubly penetrating and nonpenetrating stalks with posterior innervation (DPNP). The electrocyte phenotype of the most recent common ancestor of *Mormyrops* spp. remains unresolved, and this is indicated by dashed lines.

fishes, and they are sensitive to the much higher frequencies found in EODs. Mormyrids have two types of tuberous receptor organs, mormyromasts and knollenorgans, which give rise to anatomically distinct central electrosensory pathways (reviewed in Bell & Maler, 2005; Bell & Szabo, 1986). Mormyromasts function in active electrolocation: by detecting distortions in their self-generated electric signals caused by nearby objects, mormyrids are able to orient, navigate, and locate food (von der Emde, 1999). Knollenorgans mediate electric communication, the detection of electric signals generated by nearby fish. They are more sensitive than mormyromasts, and thus better able to detect the EODs of distant fish (Bell, 1990). In addition, corollary discharge-mediated inhibition blocks responses to the fish's own EOD at the first stage of knollenorgan processing in the hindbrain (Bell & Grant, 1989). Thus, the knollenorgan sensory system is dedicated to detecting and analyzing electric communication signals.

Knollenorgans are embedded within the epidermis. Each organ consists of a chamber that typically contains three or four sensory cells along with supporting cells at its base, and a canal filled with loosely packed epithelial cells leading to a pore on the epidermal surface (Bennett, 1965; Harder, 1968b; Jørgensen, 2005; Szabo, 1965; Zakon, 1986). Each sensory cell is large, from 40 to 50 μm in diameter, and all of the sensory cells within a knollenorgan are innervated by a single afferent nerve fiber.

The locations of knollenorgans on the body surface vary among species (Carlson et al., 2011; Harder, 1968a; Lavoué et al., 2004, 2010). In the subfamily Mormyrinae, species within "clade A" (Figure 5) have knollenorgans distributed all over the head and throughout the dorsal

and ventral surfaces of the trunk (Figure 6A). Within the subfamily Petrocephalinae, however, only two species fit this pattern. All other petrocephalines have just three clusters of knollenorgans called "rosettes" located on each side of the head (Figure 6B). The individual knollenorgans within rosettes are relatively large, containing up to 40 sensory cells (Harder, 1968a). The basal mormyrine genus *Myomyrus* has an intermediate pattern, with a single rosette located towards the back of the head along with a relatively low density of knollenorgans found on the rest of the head, as well as the dorsal and ventral surfaces of the trunk (Figure 6C). These differences are related to evolutionary change in the central knollenorgan electrosensory pathway (see below), as well as perceptual differences in the ability to discriminate variation in EOD waveform (Carlson et al., 2011). Species with a broad distribution of knollenorgans can detect temporal variation in EOD waveform, whereas species with rosettes cannot.

Evolution and Development of Electroreceptors

Electroreception appears to be an ancestral vertebrate trait. The primitive lateral line system of vertebrates consisted of both mechanosensory neuromasts and ampullary electroreceptors, both of which are found in extant lampreys, cartilaginous fishes, non-neopterygian bony fishes, and aquatic amphibians (Bullock et al., 1983; New, 1997; Rose, 2004). However, ampullary electroreceptors were lost in neopterygian bony fishes, the lineage that gave rise to teleosts. The electroreceptors of teleosts, including mormyriforms and their close relatives the xenomystins, as well as siluriforms and gymnotiforms, are therefore derived.

Primitive ampullary electroreceptors, neuromasts, and their associated lateral line nerves all arise from dorsolateral placodes, which develop within the inner layer of the cephalic ectoderm after neurulation (Gillis et al.,

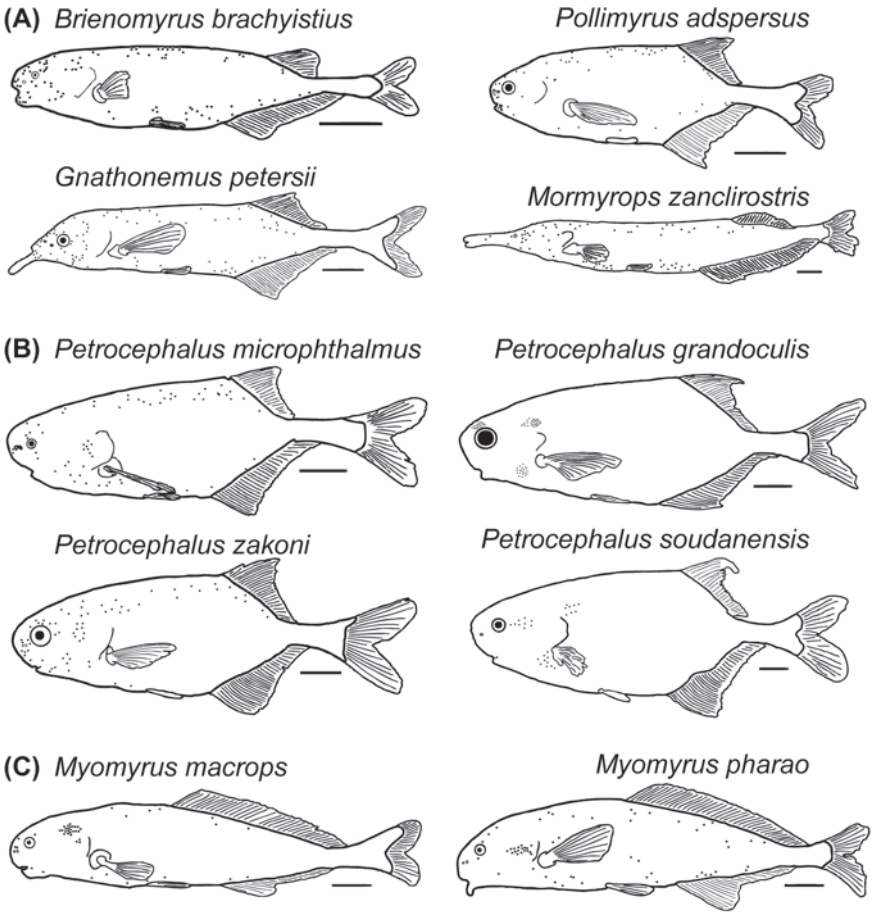

Figure 6. The locations of knollenorgan electroreceptors on the body surface vary among mormyrids. The locations of individual knollenorgans are indicated by black dots. (**A**) All species studied in clade A have knollenorgans distributed throughout the head and on the dorsal and ventral body surfaces. (**B**) Two petrocephaline species, *Petrocephalus microphthalmus* and *P. zakoni*, have a similar distribution of knollenorgans, whereas all other petrocephaline species studied have knollenorgans limited to three distinct clusters on the head called rosettes. (**C**) Species in the basal mormyrine genus *Myomyrus* have an intermediate phenotype, with a single rosette towards the back of the head, and a low-density distribution of receptors on the dorsal and ventral body surfaces. Modified from Carlson et al. (2011).

2012; Modrell et al., 2011a; Northcutt et al., 1995). Each placode follows a unique pattern of elongation or migration that establishes a complex distribution of receptors across the body surface (reviewed in Northcutt, 1989, 2005). For most of the lateral line placodes, mitotic activity leads to elongation and the formation of sensory ridges. The cells within each ridge then begin to differentiate, forming neuromast mechanoreceptor primordia within their central zone and ampullary electroreceptor primordia within their lateral zone, each originating from different cell lineages (Northcutt et al., 1994). All of the placodes express several genes that appear to constitute a regulatory network for the specification of different groups of placodes and their sensory cells (Baker & Bonner-Fraser, 2001; Freitas et al., 2006; Gibbs & Northcutt, 2004; Metscher et al., 1997; Modrell & Baker, 2012; Modrell et al., 2011a, 2011b; O'Neill et al., 2007; Schlosser, 2002).

The variation in the spatial distributions of knollenorgans among mormyrids presents a wonderful opportunity to determine how evolutionary change in developmental patterning shapes divergence in peripheral sense organs (Figure 6). Unfortunately, we know much less about the development of derived electroreceptors, and nothing is currently known about the genetic basis of their development. Derived electroreceptors share a number of anatomical and functional similarities with primitive electroreceptors (Northcutt, 2005), and they are innervated by afferent axons that enter the brain through lateral line nerves (Bell & Maler, 2005). Further, derived electroreceptor primordia develop at the lateral edges of neuromast lines from a distinct cell lineage, similar to primitive electroreceptors (Northcutt, 2005; Vischer, 1989a, 1989b). Finally, like the primitive electrosensory system, it is clear that derived electrosensory systems are ontogenetically and phylogenetically related to the mechanosensory lateral line system (Bell & Maler, 2005; Bodznick & Montgomery, 2005), which does arise from placodes in teleosts (Ghysen & Dambly-Chaudiére, 2004). These many similarities suggest at least some overlap in developmental gene regulatory networks for primitive and derived electroreceptors. However, it remains unclear whether derived electroreceptors arise from placodes or from afferent nerve induction of general ectoderm (Northcutt, 2005). Compelling, but circumstantial, evidence for the latter comes from both observations of development and experimental regeneration studies (Bever & Borgens, 1991a, 1991b; Denizot & Libouban, 1985; Roth, 1986, 1993; Vischer, 1989a, 1989b, 1995; Vischer et al., 1989). Resolving this issue will require in vivo fate mapping of lateral line placodes in species having derived electroreceptors (Northcutt, 2005), similar to the recent work that confirmed placodal origins for primitive electroreceptors in both cartilaginous and non-neopterygian bony fishes (Gillis et al., 2012; Modrell et al., 2011a).

In mormyrids, electroreceptors start to differentiate about 6 days after fertilization and they are functional within a few days later (Bensouilah et al., 2002; Denizot et al., 1998; Postner & Kramer, 1995). Five distinct electroreceptors can be recognized in developing larvae (Denizot et al., 1998). Three of these are found only in larvae, two of which degenerate at the onset of the juvenile stage concurrent with degeneration of the larval electric organ (Bensouilah et al., 2002). A third larval electroreceptor is a promormyromast that gives rise to the adult mormyromast through differentiation (Denizot et al., 2007). The remaining two electroreceptors found in larvae are the ampullary organs and knollenorgans that remain in adults. New tuberous electroreceptors can arise by budding off of old receptors, at least in the gymnotiform *Sternopygus* (Zakon, 1984).

Peripheral Coding of Electric Signals

The broadly distributed knollenorgans of clade A species (Figure 6A) contain spiking receptor cells (Bennett, 1965). Their spiking activity can be monitored noninvasively in a restrained fish by placing an electrode next to the receptor pore, and then using this electrode to directly stimulate the receptor organ and record the resulting spiking responses (Arnegard et al., 2006; Hopkins & Bass, 1981; Lyons-Warren et al., 2012). Knollenorgans respond to rapid positive voltage transients with a time-locked, short-latency (~100 μs) spike (Bennett, 1965; Hopkins & Bass, 1981). They are alternating current (AC)-coupled, which means they will respond to the onset of positive voltage steps, as well as the offset of negative voltage steps. In response to the EOD of a neighboring fish, one side of the body will experience an inward current, whereas the opposite side of the body will experience an outward current (Figure 1C). As a result, knollenorgans on opposite sides of the body receive opposite polarity signals, causing one of them to spike at the start of the EOD and the other to spike at the end of the EOD (Hopkins & Bass, 1981), thereby encoding EOD waveform into receptor spike timing differences (Figure 7A). As the relative position of the signaling fish changes, the receptors that respond to the start and end of the EOD will also change (Figure 7B). Thus, these spike timing differences also encode the location and orientation of the sender.

The IPIs of signaling fish are also encoded into knollenorgan spike times. The shortest IPIs generated by mormyrids are ~10 ms (Carlson, 2002a). This represents a firing rate of ~100 Hz, and knollenorgans are able to reliably follow stimulation rates as high as 500 Hz (Bell & Grant, 1989). Knollenorgans in clade A species therefore represent electric communication signals using a multiplexed temporal code, with spike timing differences between receptors coding for EOD waveform and sender

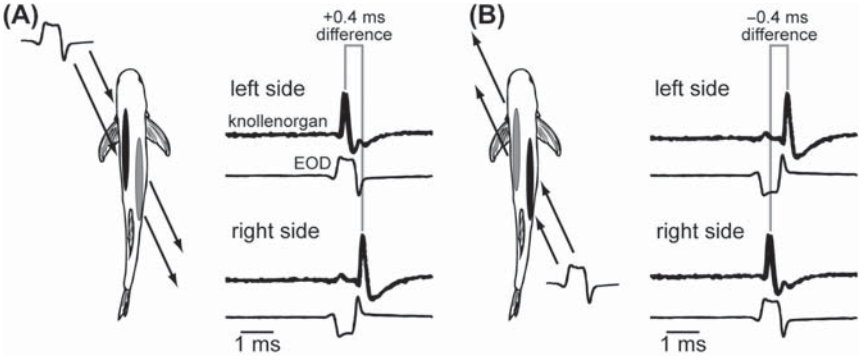

Figure 7. Knollenorgans in clade A species encode electric signals into precisely timed spikes. (**A**) Electrosensory stimulation and knollenorgan responses to a *Paramormyrops* sp. "VAD" EOD originating from the left anterior end of the receiving fish. Current flows into the left side of the body (black ellipse) and out the right (gray ellipse), causing knollenorgans on the left to receive a normal polarity EOD and knollenorgans on the right to receive a reversed polarity EOD. Each knollenorgan responds to positive voltage changes, and this difference in polarity results in a spike timing difference between knollenorgans on the left and right sides. (**B**) When the EOD comes from the opposite direction, the flow of current is reversed, causing the difference in spike timing to reverse. Thus, spike timing differences between receptors encode EOD waveform and sender location (modified from Hopkins & Bass, 1981).

location, and interspike intervals coding for IPIs (Baker et al., in press).

We know very little about the physiology of the large, rosette-type knollenorgans of petrocephalines (Figure 6B). As opposed to the spike-like activity of clade A knollenorgans, rosette-type knollenorgans appear to generate continuous oscillations at frequencies as high as 3 kHz (Harder, 1968a). However, we do not yet know how they encode electric communication signals.

Electric Signals Are Analyzed by a Dedicated Sensory Pathway

The central knollenorgan sensory pathway is illustrated in Figure 8A. Knollenorgan primary afferent fibers terminate ipsilaterally onto large, adendritic, spherical neurons in the hindbrain (Bell & Russell, 1978; Szabo & Ravaille, 1976). These cells also receive inhibitory input from the corollary discharge pathway, which blocks responses to the fish's own EODs (Bell & Grant, 1989; Mugnaini & Maler, 1987b). Convergence of multiple knollenorgan afferents onto individual postsynaptic neurons may increase the precision of temporally coded stimulus information (Baker et al., in press), which is then relayed bilaterally to the midbrain exterolateral nucleus (EL). The central knollenorgan pathway of clade A species has been intensively studied in recent years, and we are starting to decipher the underlying neural network responsible for the analysis of spike timing differences that code for EOD waveform (Amagai, 1998; Amagai et al., 1998; Friedman & Hopkins, 1998; Lyons-Warren et al., in review; Mugnaini & Maler, 1987a) and spike timing sequences that code for IPIs (Carlson, 2009; George et al., 2011; Ma et al., in review). Details on the anatomy and physiology of the knollenorgan sensory pathway, and the central analysis of spike timing differences and spike timing sequences, have recently been reviewed by Baker et al. (in press).

From a neuroethological perspective, one of the major advantages of mormyrids as a model system is the dedicated role of the knollenorgan sensory pathway in processing electric communication signals. As a general rule, a given sensory region serves multiple functions. For example, consider primate visual or auditory cortex. These regions clearly play important roles in processing communication signals, but also in a wide variety of other behavioral tasks. It is certainly possible to characterize the coding of naturalistic stimuli, but it can be difficult to relate specific features of neural circuits to behavior when those circuits serve multiple functions. Another major advantage is that the knollenorgan sensory system uses precisely timed spikes to encode stimulus information (Figure 7). This makes it easy to recreate behaviorally relevant patterns of neural activity in vitro and relate the processing of that activity to the coding of sensory information in vivo (George et al., 2011; Ma et al., in review).

Evolution and Development of Electrosensory Pathways

Both primitive and derived electrosensory pathways follow a similar anatomical organization to mechanosensory lateral line pathways (Bell & Maler, 2005; Bodznick & Boord, 1986; Finger, 1986; Montgomery et al., 1995; Northcutt, 1986). The independently derived electrosensory systems of teleosts are remarkably similar, and this may partly reflect their shared phylogenetic origins in homologous lateral line systems (Finger et al., 1986). On the other hand, these similarities are also probably shaped

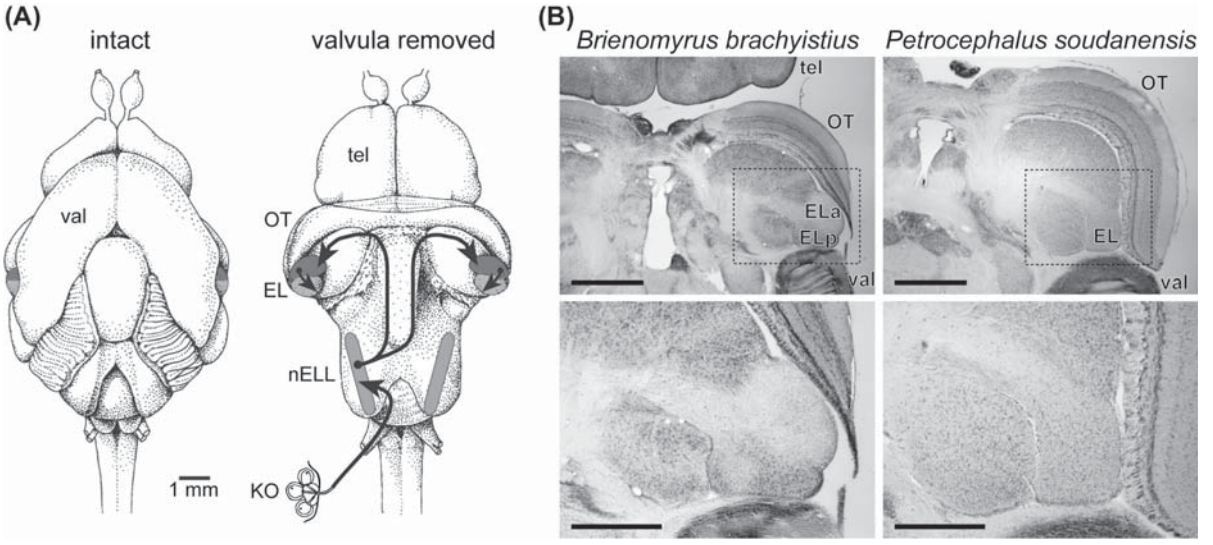

Figure 8. Evolutionary change in the knollenorgan sensory pathway. (**A**) The knollenorgan pathway to the midbrain in the clade A *Brienomyrus brachyistius*. A dorsal view of the brain is shown intact (left) and with the large valvula cerebellum removed (right). The afferent fibers of knollenorgans (KO) project through the lateral line nerves to the hindbrain, terminating ipsilaterally in the nucleus of the electrosensory lateral line lobe (nELL). Neurons in nELL project bilaterally to the midbrain exterolateral nucleus (EL), where spike times generated at the periphery are first analyzed. OT = optic tectum; tel = telencephalon; val = valvula (modified from Carlson, 2009). (**B**) Fifty-micrometer horizontal sections through the midbrain of the clade A *B. brachyistius* and the petrocephaline *P. soudanensis* stained with cresyl violet. Dashed boxes in the upper images (scale bars = 1 mm) correspond to the enlarged images below (scale bars = 500 μm). All species in clade A that have been studied, as well as *Petrocephalus microphthalmus*, have an enlarged EL divided into anterior and posterior subdivisions (ELa and ELp, respectively). Every other petrocephaline, as well as *Myomyrus* spp., has a relatively small EL lacking any apparent subdivisions (modified from Carlson et al., 2011).

by parallel or convergent evolution in response to similar computational challenges (Hopkins, 1995).

In mormyrids, species differences in the spatial organization of knollenorgans (Figure 6) are associated with evolutionary change in the central knollenorgan pathway (Carlson et al., 2011). In all clade A species, the EL brain region is relatively large and subdivided into anterior and posterior subdivisions (Figure 8B). By contrast, *Myomyrus* spp. and all but one petrocephaline species have a relatively small, undifferentiated EL (Figure 8B). Only one known species of Petrocephalinae, *Petrocephalus microphthalmus*, has an enlarged ELa/ELp. Thus, all known species that have an enlarged ELa/ELp also have broadly distributed knollenorgans (Carlson et al., 2011), though the converse is not quite true: *Petrocephalus zakoni* has broadly distributed knollenorgans and an EL (Figure 6B), and *Myomyrus* spp. have an intermediate knollenorgan phenotype and an EL (Figure 6C). Phylogenetic reconstruction suggests that EL is the ancestral character state, and that ELa/ELp is independently derived in clade A and *P. microphthalmus* (Figure 5) (Carlson et al., 2011). In all species studied, those with an ELa/ELp can detect EOD waveform variation, whereas those with an EL cannot (Carlson et al., 2011).

The neural circuitry in ELa/ELp has been well studied in a few clade A species (Baker et al., in press; Xu-Friedman & Hopkins, 1999). ELa consists of two distinct cell types, Large Cells and Small Cells, both of which receive excitatory input from ascending hindbrain axons (Figure 8A). These axons synapse onto Large Cells shortly after entering ELa, and then follow a long and convoluted path, synapsing onto Small Cells along the way (Friedman & Hopkins, 1998). These elongated axons are strongly suggestive of axonal delay lines that cause excitatory input to Small Cells to be delayed by different amounts, similar to the delay lines found in auditory sound localization circuits that are also dedicated to submillisecond temporal analysis (Ashida & Carr, 2011). Large Cells provide γ-aminobutyric acid-(GABA)ergic inhibitory input to Small Cells through a relatively direct axonal projection (Friedman & Hopkins, 1998; George et al., 2011; Mugnaini & Maler, 1987a). Thus, Small Cells respond selectively to particular spike timing differences generated at the periphery (Figure 7) by integrating delayed excitation and direct inhibition from different receptive fields (Friedman & Hopkins, 1998; Lyons-Warren et al., in review). In this way, the population of Small Cells act as time comparators for discriminating variation in EOD waveform. Small Cells provide the only projections from ELa to ELp (Friedman & Hopkins, 1998), where Multipolar Cells integrate Small Cell output and thereby respond selectively to variation in EOD waveform, amplitude, and orientation (Amagai, 1998), as well as IPIs (Carlson, 2009). For additional

details on the ELa/ELp microcircuit and the processing of spike timing differences that represent EOD waveform and spike timing sequences that represent IPIs, see Baker et al. (in press).

We currently know nothing about the anatomy, physiology, or microcircuitry of EL. Which of the three cell types found in ELa/ELp (Small Cells, Large Cells, and Multipolar Cells) are found in EL? Are there cell types in EL that are not found in ELa or ELp? How are the cells in EL wired together? Is there GABAergic inhibition? Are there axonal delay lines? Determining how the different types of knollenorgans encode electric signals and how these signals are processed in EL vs. ELa/ELp offers the promise of reconstructing evolutionary change in the brain at the level of detailed neural circuitry, and revealing the exact neural mechanisms responsible for species differences in sensory perception and communication behavior. We also know nothing about the microcircuitry of the independently derived ELa/ELp of *P. microphthalmus*. Comparing the ELa/ELp of clade A and *P. microphthalmus* provides a unique opportunity to ask whether parallel evolutionary change in neuroanatomy and sensory perception is realized by similar changes in neuronal circuitry and sensory coding.

Developmental studies of the knollenorgan sensory system would be extremely informative in understanding evolutionary change in this system. What determines the locations of knollenorgans on the body surface? What is the relative timing of knollenorgan, EL, ELa, and ELp development? Do species differences in the sensory input provided by knollenorgans guide the development of EL vs. ELa/ELp, and if so, how? Unfortunately, we currently know very little about the development of central electrosensory pathways (reviewed in Northcutt, 2005).

ELECTRIC COMMUNICATION AND DIVERSIFICATION

Animal Communication and Speciation

Speciation is the process by which new species arise. Arguably, the most important insights into mechanisms of speciation have come from lineages that have evolved exceptionally high species diversity over relatively short periods of time, so called "species radiations" (Schluter, 2000). When these radiations happen within a limited geographic range, they are referred to as "species flocks." Species radiations are frequently associated with high diversity in communication systems, which have been described for nearly every known sensory modality (Allender et al., 2003; Boul et al., 2006; Diamond, 1986; Mendelson & Shaw, 2005; Mullen et al., 2007; Sullivan et al., 2002). This connection impressed upon Mayr (1963), "If we were to rank the various isolating mechanisms of animals according to their importance, we would have to place behavioral isolation far ahead of all others." It is widely accepted that disruptive natural selection acting on divergent phenotypes is responsible for most species radiations, and therefore communication signals and other prezygotic isolating mechanisms evolve as a means of reinforcing divergence (Dobzhansky, 1937). However, this does not appear to be the case in systems where the signature of ecological divergence preceding signal divergence is low (Allender et al., 2003; Arnegard et al., 2010a; Mendelson & Shaw, 2005), suggesting the intriguing alternative hypothesis that divergence in courtship signaling behavior may itself contribute to the speciation process through sexual selection (Panhuis et al., 2001).

Evolutionary Scenarios for Mormyrid Diversification

Stoddard (1999, 2002) suggested that the evolution of EOD waveform complexity in gymnotiform fishes might have been a response to selection pressure from electroreceptive predators. Ampullary electroreceptors are sensitive to low frequencies (Zakon, 1986). Thus, monophasic EODs with relatively low frequency spectral content are easier for electroreceptive predators such as catfishes and large gymnotiforms to detect than multiphasic EODs having higher-frequency content. The elongated EODs of breeding males often contain elevated low-frequency components (Bass & Hopkins, 1984, 1985), raising intriguing questions about the costs of sex differences in electric signals and the evolution of honest signaling (Zahavi & Zahavi, 1997). Indeed, predatory electroreceptive catfish feed on mormyrids, and they can more easily detect the elongated EODs of breeding males (Hanika & Kramer, 1999, 2000).

Two different species flocks of mormyrids have been described, one in the genus *Campylomormyrus* in the Congo River (Feulner et al., 2008) and a second in the genus *Paramormyrops* in Gabon (Sullivan et al., 2002). Studies on the *Campylomormyrus* species flock suggest that divergent ecological selection may have fueled mormyrid diversification. Feulner et al. (2007) described six sympatric *Campylomormyrus* species that differ primarily in snout morphology and EOD duration. Variation in snout morphology likely affects the accessibility of different prey items (Feulner et al., 2007), and variation in EOD duration could establish differences in active electrolocation performance, allowing for specialization on different size classes of insect larvae (Feulner et al., 2008). Reproductively mature females prefer to associate with conspecific males, and this preference can be elicited solely by EOD playback (Feulner et al., 2009a). Feulner et al. (2009a) suggest that EOD divergence in the *Campylomormyrus* radiation reflects trophic niche

segregation, and that mate choice based on EOD duration represents a pleiotropic effect on assortative mating. This implies that sexual selection on EODs acts to reinforce divergence that initially arises through ecological adaptation.

Research on the *Paramormyrops* species flock suggests that sexual selection may play a more principal role in diversification. Unlike the *Campylomormyrus* radiation, the *Paramormyrops* radiation is characterized by highly divergent EODs, but low levels of morphological and genetic differentiation (Sullivan et al., 2002, 2004). Recently, Arnegard et al. (2010a) performed a formal comparison of rates of divergence in EOD waveform, body shape, body size, and trophic ecology in *Paramormyrops*. The results revealed that electric signals have diverged faster than traits related to ecological adaptation, suggesting that sexual selection has been a primary driver of divergence in this group. Field and laboratory playback experiments in *Paramormyrops* reveal strong preferences for species-specific EOD waveforms (Arnegard et al., 2006; Hopkins & Bass, 1981).

Studies of mormyrid species flocks seem to suggest that both ecological adaptation and sexual selection can contribute to the diversification of species and electric signals. Speciation may be most rapid when a trait that is subject to divergent natural selection also influences assortative mating (Servedio et al., 2011). From this perspective, the dual role of EODs in mate choice and active sensing may have promoted speciation in mormyrids (Feulner et al., 2009b).

How Does EOD Diversity Evolve?

Regardless of the exact evolutionary processes underlying mormyrid diversification, it is clear that electric signaling is a critical part of the process. Determining how selection on electric signals relates to speciation relies considerably on connecting microevolutionary, population-level processes to macroevolutionary patterns of signal evolution. Several studies have sought to describe population-level variation in mormyrid EODs (Arnegard et al., 2005; Gallant et al., 2011; Kramer & van der Bank, 2000; Kramer et al., 2003, 2004; Lamml & Kramer, 2006, 2007). We focus here on two particular lineages within the *Paramormyrops* radiation that may provide insights into macroevolutionary patterns of phenotypic change in mormyrids.

The *Paramormyrops magnostipes* complex contains three signal "morphs," two of which (Types I and II) exhibit considerable differentiation in EOD waveform, giving the appearance that the EODs are "inverted" relative to each other (Figure 1A) (Arnegard et al., 2005). This variation is likely related to between-morph differences in electric organ anatomy, particularly in the diameter of penetrating stalks (C. D. Hopkins, pers. comm.). Type I and Type II morphs occur sympatrically in several localities throughout Gabon, such as in tributaries of the Ivindo River in the north, and tributaries of the Ngounié River in the south. Arnegard et al. (2005) demonstrated that sympatric morphs are genetically identical across five microsatellite loci, suggesting that waveform differentiation may have occurred despite substantial gene flow and that the different morphs may represent incipient species. The two morphs overlap substantially in both trophic ecology and morphology (Arnegard et al., 2010a), suggesting that sexual selection on EODs, rather than ecological adaptation, may be driving this phenotypic divergence. Recordings from knollenorgan electroreceptors and playback experiments reveal sensory and behavioral discrimination of the different EOD waveforms (Arnegard et al., 2006).

Paramormyrops kingsleyae, in contrast with *P. magnostipes*, exhibits clinal variation in EOD waveforms among geographically isolated populations (Gallant et al., 2011). *P. kingsleyae* are polymorphic for Pa- or NPp-type electric organs, which produce P0-present (triphasic) and P0-absent (biphasic) EODs, respectively. P0-absent populations are relatively rare, and probably originated twice in association with barriers to migration (Gallant et al., 2011). In one case, a waterfall (Bongolo Falls) appears to act as a barrier, and P0-absent and P0-present individuals are found in sympatry along this boundary. Further, individuals collected from these locales had electric organs with mixed morphology, consisting of both NPp- and Pa-type electrocytes. Thus, the two signal types, separated by the waterfall, may hybridize when seasonal flooding temporarily connects the two watersheds. There is also clinal variation in EOD duration in *P. kingsleyae* (Gallant et al., 2011). The variation in EOD duration is relatively minor, and when P0 is present, it is small compared with the P0 of other species, suggesting that intraspecific variation in *P. kingsleyae* does not result from strong selection. Instead, Gallant et al. (2011) hypothesized that EOD variation in *P. kingsleyae* is related to genetic drift and patterns of gene flow between allopatric populations, which is influenced by drainage patterns and barriers such as waterfalls and oceans.

Both species, *P. magnostipes* and *P. kingsleyae*, capture aspects of EOD diversity within populations that reflect broader patterns of diversity within the *Paramormyrops* radiation and among mormyrids in general. For example, reversals from Pa- to NPp-type electrocyte morphology such as those occurring in *P. kingsleyae* are common in mormyrids, particularly within the *Paramormyrops* (Sullivan et al., 2000, 2004). Based on these frequent reversions, as well as the existence of "hybrid" individuals in *P. kingsleyae*, we have hypothesized that the presence/absence of penetrating stalks may have a simple genetic basis (Gallant et al., 2011). Both *P. kingsleyae* and *P. magnostipes* suggest that EOD diversification can take place relatively rapidly in the face of ongoing gene flow and

without any obvious signatures of ecological divergence. *P. magnostipes* suggests that strong sexual selection may drive signal divergence in sympatry, whereas *P. kingselayae* highlights the effects of geographic isolation on patterns of signal divergence. Together, these two processes have likely contributed substantially to the evolution of EOD diversity among mormyrids.

Novelty, Innovation, and Diversification

The active electrolocation and electrocommunication systems of mormyrids are clearly evolutionary novelties (Pigliucci, 2008). Further, they fit the definition of being key innovations that have fueled diversification by opening up new ecological niches and new channels of communication, respectively (Carlson & Arnegard, 2011; Hunter, 1998; Schluter, 2000; Simpson, 1953). Mormyrids are a highly successful lineage of an ancient teleost clade, constituting over 90% of extant Osteoglossomorph species diversity (Carlson & Arnegard, 2011; Eschmeyer & Fricke, 2011). Clade A is responsible for over 80% of mormyrid diversity (Carlson et al., 2011), and the only two identified Osteoglossomorph species flocks are both in clade A (Feulner et al., 2008; Sullivan et al., 2002). Thus, clade A is clearly a "hotbed" of mormyrid diversification, and this is linked to two specific neural innovations: the origin of penetrating electrocytes in the Mormyrinae and the origin of ELa/ELp in clade A (Figure 5). The evolution of developmental mechanisms for modifying the stalk structure of electrocytes increased the potential for generating EOD waveform diversity (Sullivan et al., 2000). Evolutionary change in the knollenorgan sensory system allowed for the perceptual detection and discrimination of this variation (Carlson et al., 2011). Together, these two neural innovations increased the "signal space" available for electric communication, affording greater potential for the diversification of EODs (Carlson, 2012). Regardless of the exact mechanisms underlying evolutionary divergence in mormyrids, it is clear that electric communication has been a key player. The evolution of novel motor and sensory abilities in clade A has therefore fueled a 3- to 5-fold increase in species diversification rates, and a greater than 10-fold increase in the rates of EOD waveform evolution (Carlson et al., 2011).

FROM GENES TO BEHAVIOR: A CALL TO PIPETTES

Mormyrid research has "sparked" half a century of insights into neuroscience and evolutionary biology, and has provided some tantalizing glimpses into the evolution and development of novel sensory and motor systems. To capitalize on the advantages of this system and truly gain fundamental insight into evolutionary developmental mechanisms of behavioral diversification, the next half-century of mormyrid research must embrace the genomics revolution. A key resource for progress on this research agenda is the establishment of genomic and transcriptomic resources. A call for genomic sequencing in gymnotiform electric fishes has been made (Albert et al., 2008). Here, we make the case for genomic sequencing in mormyrids. The unique strengths of mormyrids for linking morphology and physiology to behavior have been firmly established. Now is the time to capitalize on these advantages and decipher the genetic basis of behavioral diversification to answer questions broadly relevant to biology.

The mormyrid genome, estimated to be 1–1.2 Gb in size (Hinegardner & Rosen, 1972), is a strategically valuable sequencing candidate for the broad fields of genome biology and evolutionary biology. To date, all sequenced teleost fishes are euteleosts (as are gymnotiform electric fishes). By contrast, mormyrids are osteoglossomorphs, the most basal extant lineage of teleost fishes. Genome sequencing in this group would provide a critical reference point for broad evolutionary comparisons. Osteoglossomorphs are hypothesized to be the first extant lineage to split from the teleost lineage following the hypothesized fish-specific genome duplication (Hoegg et al., 2004). Thus, a complete osteoglossomorph genome would be of particular value to understanding genome duplication and its role in species diversification and the evolution of novelty.

Beyond the value to understanding the evolution of vertebrate genomes, mormyrid genomics resources will enable a new generation of studies to assess gene expression quantitatively on spatial and temporal scales, and relate it to the morphological, physiological, and developmental processes underlying behavior. Such approaches have not been applied widely in mormyrids (or other electric fish) because of the lack of genomics resources necessary to develop primers, probes, and antibodies. Antibodies and in situ probes would facilitate sorely needed developmental studies of electrosensory and electromotor pathways. Further, identified gene sequences offer a potential avenue for gaining insight into the ionic basis of EODs while circumventing the difficulties of electrophysiological recordings from mormyrid electric organs. Quantitative polymerase chain reaction (PCR) could be used to identify ion channels in the electric organ, in situ hybridization could be used to localize those channels, and heterologous expression could be used to study their physiology. In situ hybridization could also be used to determine how seasonal hormone fluctuations elicit structural changes in electric organs. Finally, a greater number of genomic loci for population genetic sampling would strengthen our understanding of the spatial and temporal relationships among mormyrid species, populations, and individuals using phylogenetics.

Another major prospect enabled by the availability of genomics resources in mormyrids is a greater understanding of the relative importance of sexual selection and natural selection in speciation. *Campylomormyrus* and *Paramormyrops* exemplify two major riverine species radiations (Feulner et al., 2007; Sullivan et al., 2002), and evidence from the two radiations point to ecological adaptation and sexual selection, respectively, as ultimate causal factors (Arnegard et al., 2010a; Feulner et al., 2008). Depending on whether divergent sexual selection or divergent natural selection acts earliest in the speciation process determines whether the earliest genomic targets of selection have influenced genes for ecological "fitness" or sexual "attractiveness." Thus, a key question that is eminently addressable by comparative genomics is whether or not the genetic architecture of speciation (i.e., the genes and their regulatory elements underlying traits acted upon by divergent selection) is similar between lineages that have radiated in response to fundamentally different selective pressures.

Although most species radiations are presumed to result from natural selection acting divergently upon traits (Schluter, 2000), *Paramormryops* and a few other model systems have established the possibility that divergent sexual selection on courtship signals may be responsible for at least some radiations. Indeed, a recent comprehensive study of cichlid fishes suggests that both ecological opportunity and sexual selection acting on mating signals have been critical factors in determining the extent of diversification in different lineages (Wagner et al., 2012). Because of detailed morphological, physiological, and behavioral studies, we have an unparalleled understanding of the salient features of mormyrid communication signals and how they are generated by their motor system and perceived by their sensory system (reviewed in Baker et al., in press; Carlson, 2002a, 2006; Hopkins, 1986a). In this sense, comparative genomics studies would be invaluable in elucidating the genetic basis of traits associated with diversification of communication systems, such as signal variation, signal perception, and signal preferences, in the context of mate choice. Most tractable in this regard may be studies of species with multiple morphs, such as *Paramormyrops kingsleyae* (Gallant et al., 2011) and *P. magnostipes* (Arnegard et al., 2005), which are divergent in signal features characteristic of broader patterns of phylogenetic diversity. Genome sequencing in these groups would greatly facilitate high-density linkage maps for quantitative trait mapping.

A third major prospect enabled by the availability of genomics resources in mormyrids is the potential to examine signatures of selection and identify where, how strongly, and in what direction selection has acted. Mormyrid fishes have evolved both a novel sensory system (an ancestral vertebrate trait that was lost and then regained twice independently) and novel motor system (a derived vertebrate trait that has evolved at least six times independently), both of which likely involve hundreds or thousands of proteins working in concert. We currently understand the strength and direction of selection on only one mormyrid gene, the sodium channel *scn4aa* (Arnegard et al., 2010b; Zakon et al., 2006, 2008). Comparative genome sequencing between mormyrids and other teleosts may elucidate signatures of selection in the genome related to the evolution of these novel systems. Similarly, comparative genome sequencing among mormyrids may elucidate genetic mechanisms underlying the morphology and distribution of knollenorgans (Figure 6), the evolution of ELa/ELp (Figures 5, 8), and the origins of diversity in electrocyte morphology (Figure 5). Not only would such approaches help reveal how these structures might have evolved, they would also point to a genetic basis for morphological and physiological features that have already been related to specific behaviors and perceptual abilities. Identification of such genes would also facilitate the development of genetic manipulation tools such as RNAi to provide experimental verification of how these traits determine behavior.

Evolutionary change in *cis*-regulatory elements, changes to protein coding sequences, and neofunctionalization of gene duplicates are all plausible hypotheses underlying the evolution of novel phenotypes. There is support for each of these mechanisms, but systematic comparative studies that differentiate the relative contributions of these mechanisms to evolutionary change in closely related species are lacking (Hoekstra & Coyne, 2007). Combining analysis of genomic signatures of positive selection with comparative transcriptome sequencing would permit the identification of regulatory elements undergoing positive selection, and it would allow for a direct comparison of the relative importance of lineage specific changes in gene expression vs. gene sequences in mediating phenotypic change. Combined analyses of the molecular underpinnings of specific mormyrid phenotypes may eventually clarify the relative contribution of each of these mechanisms to the evolution of novelty, a major goal in the broader fields of evolutionary and developmental biology (Pigliucci, 2008; Shubin et al., 2009; Wagner & Lynch, 2010). Importantly, several behaviorally important morphological phenotypes have evolved in parallel in different mormyrid lineages. Comparative transcriptomics and genomics are especially powerful at identifying the genetic basis of phenotypes when they can be applied to lineages that have independently evolved those phenotypes, helping to separate phenotypic signal from phylogenetic noise.

The field of evo-devo has focused largely on the genetic and developmental mechanisms underlying morphological variation (Carroll, 2008). It is unclear whether the insights are generalizable for understanding evolutionary change in other aspects of phenotype,

such as physiology and behavior. Mormyrids, by virtue of having well-understood relationships between morphology, physiology, and behavior, offer the opportunity to develop deeply integrative studies on the evolution, development, and control of behavior, but only if genomic and transcriptomic resources can be developed. Genomic resources are essential to bring the considerable strengths of the mormyrid system to bear on questions fundamental to biology, such as understanding the genomic architecture of speciation under various types of divergent selection, identifying the substrates underlying the evolution and development of novel phenotypes, and describing the genetic basis for species differences in behavior and its underlying mechanisms. The identification of these substrates and their contributions to behavioral variation and species divergence will paint a comprehensive picture of how selection acts on complex phenotypes such as physiology and behavior to create novelty and diversity.

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