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Electric fish genomics: Progress, prospects, and new tools for neuroethology

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ABSTRACT

Electric fish have served as a model system in biology since the 18th century, providing deep insight into the nature of bioelectrogenesis, the molecular structure of the synapse, and brain circuitry underlying complex behavior. Neuroethologists have collected extensive phenotypic data that span biological levels of analysis from molecules to ecosystems. This phenotypic data, together with genomic resources obtained over the past decades, have motivated new and exciting hypotheses that position the weakly electric fish model to address fundamental 21st century biological questions. This review article considers the molecular data collected for weakly electric fish over the past three decades, and the insights that data of this nature has motivated. For readers relatively new to molecular genetics techniques, we also provide a table of terminology aimed at clarifying the numerous acronyms and techniques that accompany this field. Next, we pose a research agenda for expanding genomic resources for electric fish research over the next 10 years. We conclude by considering some of the exciting research prospects for neuroethology that electric fish genomics may offer over the coming decades, if the electric fish community is successful in these endeavors.

Introduction

The powerful tools of genomics and genome manipulation are currently used nearly exclusively on a handful of well-established model species whose facile genetics and tractable husbandry allowed for the development of those tools. While this drives progress in those fields, the paucity of systems with reliable genomic data limits the insights that can be gained into the genetics of ecologically relevant traits, and may impede work on a number of central evolutionary problems. A core strength of the electric fish research, and of neuroethology as a discipline, is the focus on phenotype, broadly construed. This focus, combined with the strengths of electric fish as a model system, places neuroethology in a position to contribute to three of the five 'Grand Challenges' for biology as recently set out by the National Research Council (National Research Council, 2010) namely Connecting Genotype to Phenotype, Understanding the Brain, and Understanding Biological Diversity.

Since the discovery that weakly electric fish use electricity to sense their surroundings and communicate (Lissmann, 1958), researchers with interests and expertise spanning the range of biological disciplines have congregated around electric fish. Although the focus of contemporary work in the field has broadened considerably, electric fish researchers have continued to make valuable phenotypic and ecological connections that outpace many model organisms. The combination of genomics and the unique physiology of electric fishes – where the details of the electrosense link ecology and evolution intimately with neuroanatomy and ion channel kinetics (Fig. 1) – could allow for sweeping insights into how genotype connects to phenotype in an ecologically relevant system.

In the last few years, the availability of low-cost, high-throughput next-generation sequencing and sophisticated new molecular genetic techniques has laid the foundations for a 'genomic renaissance' in electric fish research. This paper will review the work of molecular biology in electric fish, beginning with the early biochemical contributions of *Torpedo* and *Electrophorus* and ending with whole genome sequencing efforts. In this work, the electric organ discharge (EOD) is a window into the neural system and the molecular workings of the electric organ (EO) and its component cells, and a view outward onto the ecology, behavior and evolution of the whole organism (Fig. 1). The latter part of the paper will then discuss the methods and benefits of integrating genomic and molecular tools into existing research programs.

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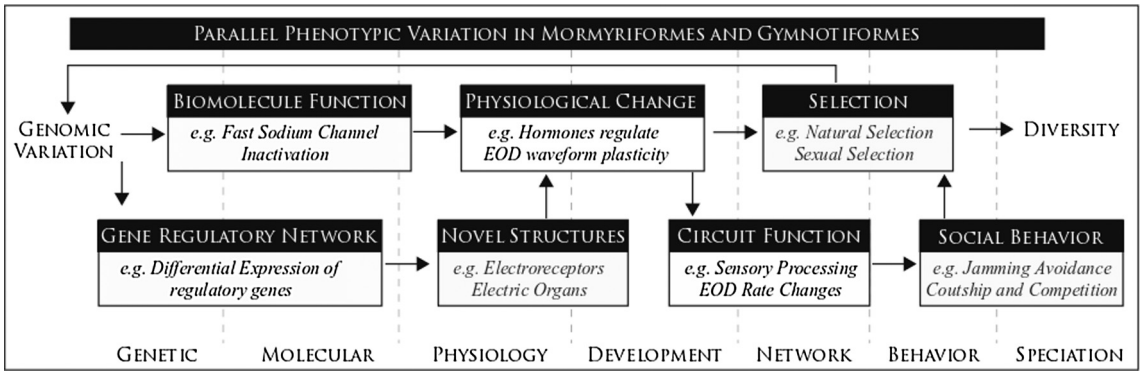

Fig. 1. The 'integrated phenotype concept' in electric fish.

1. Molecular biology of electric fishes: the first 30 years

1.1. The molecular biology of the neuromuscular junction

Torpedo rays (Miledi et al., 1971; and to a lesser extent *Electrophorus electricus*; Changeux et al., 1970) contributed deeply to our understanding of synaptic transduction. With their large EOs – *Torpedo* is "essentially a swimming purified acetylcholine receptor" (Miller, 2000) – these species provide researchers with the abundant source of receptor-rich membranes needed for describing the structure and biophysical properties of proteins at the neuromuscular junction (NMJ). Given the connection between EO and motor plates (Keesey, 2005), researchers could extract and purify the nicotinic acetylcholine receptor (nAChR) with relative ease (Sobel et al., 1977). These early biophysical studies were inherently comparative: immunohistological, ultrastructural (Rieger et al., 1976), and functional (Hess et al., 1982); however their efforts culminated in studies describing the detailed nanostructure of the nAChR (Kistler and Stroud, 1981), visualization of the molecular subunits forming the 'rosette' around the ionophoretic channel (Kistler et al., 1982), and finally a full 3D model of the molecule (Mitra et al., 1989). In addition, this research lead to the characterization of a number of other important proteins at the NMJ – notably agrin (Nitkin et al., 1987), dynein (Mou et al., 1998), and rapsyn (Elliott et al., 1980).

Beginning in 2007, Nazarian et al. (2011, 2007) demonstrated the potential for using genomic tools to probe the identity of NMJ proteins using high throughput Sanger sequencing. Nazarian et al. (2011) built a database of NMJ-associated proteins and transcripts present in electroplaques of *T. californica*, enabling the characterization of a suite of mammalian expressed sequence tags, and proteins of unknown function, as being associated with the NMJ. These discoveries proved crucial to the fields of biophysics and biomedicine (reviewed by Changeux (2012)), but although this work is indirectly relevant to neuroethology, it is the weakly electric fishes that have received the majority of the attention from the field.

1.2. Molecular biology of the EO: effectors and modulators

EOD duration varies 100-fold among species (Hopkins, 1999). This diversity is due in large part to variation in the electrocyte Na^+ currents (Ferrari et al., 1995), which in turn are regulated by voltage-gated Na^+ channels, and these are among the best studied electrocyte proteins. Research using polymerase chain reaction (PCR), quantitative PCR (qPCR), cloning, and first-generation sequencing has provided sequences of Na^+ channel genes from around 20 species of weakly electric fishes, representing both Gymnotiformes and Mormyroids (Arnegard et al., 2010; Lopreato et al., 2001; Zakon et al., 2006). These studies provide a striking example of parallel molecular evolution: independent neofunctionalization (Ohno, 2013) of the voltage-gated Na^+ channel gene *scn4aa* in both lineages.

The teleost-specific whole-genome duplication event (Section 1.4) left these taxa with two paralogs (*scn4aa* and *scn4ab*) of the tetrapod muscle Na^+ channel gene $Na_v1.4$ (Lopreato et al., 2001; Novak et al., 2006). Following down-regulation of *scn4aa* in the ancestors of both lineages (Thompson et al., 2014), the expression of this paralog was found to be restricted to the EO (Zakon et al., 2006). Freed from the selective constraint to maintain the muscle phenotype (as *scn4ab* retained this function), *scn4aa* experienced strong positive selection on amino acid replacements critical for Na^+ channel kinetics, allowing for the evolution of EOD waveform diversification and signal complexity in both lineages (Arnegard et al., 2010; Zakon et al., 2008).

This subfunctionalization (evolutionary repurposing of duplicate genes; Magadum et al., 2013; Ohno, 2013) of membrane ion channels allows for greater EOD complexity and faster firing rates, but these properties are not fixed, even within a species. The EOD frequently varies between sexes and seasons, and is regulated by hormones (Bass and Hopkins, 1984; Hopkins, 1972). Furthermore, many studies have demonstrated that a variety of hormones have effects on the EOD (Allee et al., 2008; Bastian et al., 2001; Deemyad et al., 2013; Dulka et al., 1995; Dunlap et al., 2006; Dunlap and Zakon, 1998; Maler and Ellis, 1987; Markham et al., 2009a; Mills and Zakon, 1987; Smith and Combs, 2008; Telgkamp et al., 2007; Zupanc, 2002).

Changes to the EOD mediated by hormones have been recorded over a period of minutes (Markham and Stoddard, 2005), days or weeks (Dunlap et al., 1997; Ferrari et al., 1995; McAnelly and Zakon, 2007), and each is regulated differently. Changes occurring on the timescale of minutes are regulated by trafficking Na^+ channels into the electrocyte membrane (Markham et al., 2009b), and is circadian and socially controlled in *Sternopygus macrurus*. Circadian variation in EODs, at least in *Brachyhypopomus* spp. (Franchina and Stoddard, 1998; Stoddard et al., 2007), is probably mediated by glutamate (Silva et al., 2008) and vasotocin (Perrone, 2010). Short-term hormonal EOD modulation seems to be mediated through cAMP and PKA after G-coupled receptor activation (Markham and Stoddard, 2005; McAnelly et al., 2003; McAnelly and Zakon, 1996).

Sexual dimorphism in EODs occurs in both Gymnotiformes (Allee et al., 2009; Ho et al., 2010; Markham and Stoddard, 2013; Smith, 2013) and mormyrids (Bass and Hopkins, 1985), and while androgens appear to be involved, other factors are implicated in mediating the dimorphism (Allee et al., 2009). Sexual dimorphism appears to have diverged rapidly, at least among *Apteronotus* spp. (Ho et al., 2010). The EOD (Carlson et al., 2000; Ho et al., 2010)