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Convergent patterns of evolution of mitochondrial oxidative phosphorylation (OXPHOS) genes in electric fishes

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The ability to generate and detect electric fields has evolved in several groups of fishes as a means of communication, navigation and, occasionally, predation. The energetic burden required can account for up to 20% of electric fishes' daily energy expenditure. Despite this, molecular adaptations that enable electric fishes to meet the metabolic demands of bioelectrogenesis remain unknown. Here, we investigate the molecular evolution of the mitochondrial oxidative phosphorylation (OXPHOS) complexes in the two most diverse clades of weakly electric fishes—South American Gymnotiformes and African Mormyroidea, using codon-based likelihood approaches. Our analyses reveal that although mitochondrial OXPHOS genes are generally subject to strong purifying selection, this constraint is significantly reduced in electric compared to non-electric fishes, particularly for complexes IV and V. Moreover, analyses of concatenated mitochondrial genes show strong evidence for positive selection in complex I genes on the two branches associated with the independent evolutionary origins of electrogenesis. These results suggest that adaptive evolution of proton translocation in the OXPHOS cellular machinery may be associated with the evolution of bioelectrogenesis. Overall, we find striking evidence for remarkably similar effects of electrogenesis on the molecular evolution of mitochondrial OXPHOS genes in two independently derived clades of electrogenic fishes.

This article is part of the theme issue 'Linking the mitochondrial genotype to phenotype: a complex endeavour'.

1. Introduction

The emergence of aerobic metabolism is believed to underlie the vast diversity of form and function within animals [1]. Metabolic adaptations and plasticity have facilitated the evolution of extremely complex phenotypes and behaviour [2,3]. The foundation of this metabolic adaptation lies in the capacity of mitochondria to supply enough energy to maintain these novel functions at a cellular level [4]. Bioelectrogenesis, or the ability to send and receive electric signals, is an example of a complex sensory system that requires a considerable amount of energy [5–7]. Therefore, it serves as an excellent system to investigate molecular adaptations to elevated metabolic burden associated with novel phenotypes.

Six lineages of fishes have independently evolved specialized electric organs (EOs) that produce electric fields [8,9], mostly for communication and navigation [10], but in some cases also predation [11]. Of the electrogenic groups, the Gymnotiformes of South America, with 244 species, and the Mormyroidea of Africa, with 229 species, are the most diverse [9]. These two clades exhibit many similarities, including the evolution of species-specific signals, electric organ discharges (EODs) with high-frequency peak spectral properties, and specialized tuberous electroreceptors that are tuned to the self-generated electric field [9]. The independent evolution of bioelectrogenesis in gymnotiforms and mormyroids

makes them an outstanding system for investigating convergent patterns in molecular evolution associated with this novel sensory modality.

At the molecular level, both gymnotiform and mormyroid fishes rely on specialized voltage-gated sodium channels in the EO cells (electrocytes), to generate action potentials necessary for EOD [12,13]. The Na^+ influx that depolarizes electrocytes exceeds the Na^+ currents in other excitable cells by orders of magnitude [14,15]. In order to maintain excitability of the electrocytes, Na^+ and K^+ electrochemical gradients need to be maintained by Na^+/K^+ adenosine triphosphatase, an ATP-consuming ion pump [16]. EOs consume the same order of magnitude of ATP molecules per gram of tissue per second as other metabolically demanding organs like the brain, heart and retinas [5]. However, while the brain, heart and retinas make up only 2% of body mass of organisms [17], EOs can amount to 10% of the total body mass of electric fishes [18]. It has been estimated that up to 20% of the daily metabolic reserve of the fishes is dedicated to maintaining this sensory system [5,18].

Mitochondria are the energy suppliers of the cell, producing roughly 90% of the cellular ATP reserve. Mitochondria support the cellular energetic demands via the highly efficient chemiosmotic coupling of electron and proton transfer to ATP synthesis that takes place in the inner mitochondrial membrane (IMM) [19,20]. Within the IMM, multi-subunit oxidative phosphorylation (OXPHOS) protein complexes facilitate the translocation of protons from the mitochondrial matrix to the mitochondrial intermembrane space (IMS) where an electromotive force builds up and is then used by another OXPHOS complex, complex V, to generate ATP from ADP (see the electronic supplementary material, figure S1). OXPHOS complex I, also known as NADH : ubiquinone reductase, couples the conversion of NADH to NAD^+ with the translocation of four protons to the IMS [21,22]. Complex II is involved in the Krebs cycle, and catalyses the conversion of succinate to fumarate [23] with no coupled proton translocation. Respiratory complex III, also known as cytochrome bc_1 complex, mediates the transfer of electrons from quinol molecules to cytochrome *c*, mobile electron carrier in the IMM [24]. Respiratory complex IV, also known as cytochrome *c* oxidase, uses electrons from the electron carrier, Cyt *c*, and molecular oxygen to generate water molecules [25]. Complex V couples the translocation of protons down the concentration gradient from the IMS back to the mitochondrial matrix with rotary movement in the F_1 region to generate ATP from ADP [26]. Apart from complex II, all metazoan OXPHOS complexes contain subunits encoded by both the mitochondrial and nuclear genomes. In vertebrates, the mitochondrial genome encodes 13 core OXPHOS subunits (electronic supplementary material, figure S1). The large number of mitogenomes that have been sequenced for phylogenetic analyses provides a rich system for investigating the relationship between molecular evolution of mitochondrial DNA (mtDNA) and metabolic performance.

Comparative approaches of sequence analysis allow the estimation of selective pressures acting on protein-coding genes, by determining the rates of non-synonymous (d_N) and synonymous (d_S) substitutions [27]. Codon-based likelihood approaches take advantage of the heterogeneity of selective constraints on protein-coding genes and amino acid sites to identify amino acid residues that underlie adaptive molecular evolution [28,29]. These phylogenetically based statistical methods have been widely used to detect changes in evolutionary rates across lineages in response to ecological or evolutionary processes in wide variety of vertebrate groups [30–33], as well as functional divergence following duplication events [34]. Codon-based methods of estimating d_N/d_S are therefore powerful tools for testing hypotheses concerning the molecular changes accompanying major evolutionary events [35].

Here, we study the evolution of mitochondrial OXPHOS genes of South American and African electric fishes by implementing codon-based models to evaluate changes in selective pressures that may have accompanied the evolution of electrogenesis. We take advantage of the fact that bioelectrogenesis evolved independently in gymnotiforms and mormyroids to search for convergent patterns of evolutionary constraint and selection in OXPHOS genes across these lineages. Shared patterns are likely to indicate shared evolutionary responses to the metabolic burden of bioelectrogenesis. Because the OXPHOS system plays a crucial role in cellular energy production, OXPHOS genes are highly conserved [36,37]. We examined patterns of evolutionary constraint for the mitochondrial genes of different OXPHOS complexes, to determine whether there are consistent differences between electric and non-electric fishes. We found evidence of elevated d_N/d_S in both gymnotiforms and mormyroids compared to their close relatives, indicative of a shared pattern of reduced evolutionary constraint in electric fishes. In addition, although there is an overall pattern of strong evolutionary constraint in OXPHOS genes, we tested whether there was evidence of positive selection along the branches leading to the two electric fish clades. This would be expected if the evolution of electrogenesis in fishes was associated with adaptive evolution of OXPHOS genes. A concatenated analysis of mitochondrial genomes provided strong evidence for positive selection associated with the initial evolution of bioelectrogenesis in each clade.

2. Material and methods

(a) Sequence alignment and phylogeny

We assembled two datasets to investigate the molecular evolutionary rates of mitochondrial OXPHOS genes of, respectively, the South American gymnotiform electric fishes (South American dataset hereafter) and the African mormyroid electric fishes (African dataset hereafter) from Genbank. For the South American dataset, we aligned 95 mitochondrial genomes from gymnotiform electric fishes and close relatives (other Ostariophysian fishes) [38] in Geneious v.6 [39] using the MUSCLE [40] plug-in. The maximum-likelihood phylogenetic tree used for analyses was generated using the complete mitochondrial genome sequences (see [38] for details; electronic supplementary material, figure S2). For the African dataset, we aligned 107 complete mitochondrial genomes of mormyroid electric fishes and close relatives (other Osteoglossiformes, Clupeiformes and Elopiformes species, as well as *Lepisosteus oculatus* as an outgroup) using MUSCLE. This whole-genome alignment was used to generate a maximum-likelihood phylogeny using IQ-Tree v.1.5.4 [41] with 1000 bootstrap replicates (electronic supplementary material, figure S3) using the substitution model GTR + F + R8 determined to be the best-fit model in IQ-Tree model finder. Electronic supplementary material, table S1 provides accession numbers of all sequences used in this study.

(b) Analyses of molecular evolution

We used codon models as implemented in the codeml v. 4.8 program of the PAML4 software package [42] to estimate ratios

of non-synonymous (d_N) to synonymous (d_S) substitution rates. The d_N/d_S ratio provides evidence into selective constraint and pressures acting on protein-coding genes, with a ratio below 1 indicating purifying selection, a ratio at 1 indicating neutral evolution and a ratio greater than 1 consistent with positive selection. We employed random sites models that estimate d_N/d_S across an entire phylogeny [28], as well as branch-sites [43] and clade models [44] that allow the estimation of d_N/d_S for specific branches and clades of interest in relation to other clades and the rest of the tree.

We conducted clade model analyses [44] using CmC and CmD models as implemented in PAML4 [42], with the Gymnotiformes clade labelled as the foreground in the South American dataset and Mormyroidea as the foreground in the African dataset. Random sites analyses were conducted on the South American and African datasets, as well as on separate datasets composed of different fish orders for comparison (Gymnotiformes, Characoidei, Mormyroidea and Clupeiformes). We also compiled an expanded dataset of Gymnotiform ATP6, ATP8, CYTB and COI genes by adding additional available partial sequences from Genbank, and ran random sites analyses, as well as clade model C and D analyses with Characoidei species in the background, to ensure that our results are not biased by the under-representation of Gymnotiformes in the South American dataset, or owing to the over-representation of the Siluriformes outgroup species. Finally, we conducted analyses using branch-sites models on individual genes, as well as concatenated datasets of the 13 mitochondrial protein-coding genes for both the South American and African datasets, in order to test for positive selection of OXPHOS genes along the ancestral branches leading to electric fishes in the two datasets.

Tests of selection were conducted using likelihood-ratio tests as follows: M2a versus M1a (null), M3 versus M0 and M2a, M8 versus M7. For clade model analyses, clade model C was compared to M2a_rel as the null model [45], and clade model D was compared to M3. Branch-site model A was compared to a null model where the d_N/d_S of the positive selection site class in the foreground partition is restricted to 1.0 [43]. Support for sites under positive selection was inferred using Bayes empirical Bayes (BEB) analyses [46].

In addition, FunDi [47] was used to estimate functional divergence of the electric fish OXPHOS subunits from the non-electrogenic relatives. FunDi uses a mixture model of protein evolution, in a maximum-likelihood framework, to estimate the posterior probability of each residue in the protein to belong to a functionally divergent class. In these analyses, the electric fish clades were tested against all the remaining taxa in the respective phylogenies. Residues with a posterior probability above 0.5 were considered functionally divergent [47,48].

(c) Crystal structure analyses

To gain insight regarding functional implications of our d_N/d_S analyses, we mapped the positively selected amino acid residues from the branch-site analysis of the concatenated analysis in Chimera [49]. As the majority of positively selected residues belong to complex I, we focused our structure analysis on the crystal structure from *Ovis aries* (PDB: 5LNK). In both the South American and African datasets, we only mapped residues with high posterior probability (above 0.8) of belonging to the positively selected site class.

3. Results

(a) Similar patterns of evolutionary constraint in South American and African electric fishes

Using random sites models to investigate overall rates of d_N/d_S evolution in individual mitochondrial OXPHOS genes, we find evidence for pervasive purifying selection across the tree in both the African and South American datasets. Likelihood-ratio tests with random sites models did not provide any evidence for positive selection, with all d_N/d_S estimates found to be below 1, indicating a general pattern of strong purifying selection (see the electronic supplementary material, tables S2 and S3).

Variation was detected in the strength of this constraint among OXPHOS genes, with complexes III and IV being the most conserved, and complexes I and V having higher d_N/d_S values indicative of weaker purifying selection. ATP8 in complex V was found to have the highest d_N/d_S values across both datasets (electronic supplementary material, tables S2 and S3).

Our molecular evolutionary analyses of selection show strikingly similar patterns of d_N/d_S differences between electric and non-electric fishes across the four mitochondrially encoded OXPHOS complexes (figure 1; electronic supplementary material, table S4). For all mitochondrial OXPHOS complexes, overall d_N/d_S estimates under the M0 model are higher for electric than non-electric fishes, with the exception of complex I for the African dataset (figure 1; electronic supplementary material, table S4). The overall similarity of patterns between these two distantly related lineages is remarkable. For both Gymnotiformes (South American) and Mormyroidea (African) comparisons, complex V shows the largest difference between electric and non-electric fishes, as well as the highest overall values of d_N/d_S.

This pattern was also confirmed using clade model analyses to statistically test for significant differences in d_N/d_S between electric and non-electric fish groups in both datasets. Analyses of individual genes (CmC and CmD; [44]) demonstrated statistically significant differences in d_N/d_S between electric and non-electric fishes for most genes, with electric fishes having higher d_N/d_S values than non-electric fishes (electronic supplementary material, tables S5–S7). The one exception was CYTB, which showed electric fishes having lower d_N/d_S (electronic supplementary material, table S6). These patterns of significant changes in evolutionary constraint of OXPHOS subunits in electric fishes would be consistent with shifts in metabolic demand associated with bioelectrogenesis.

Analyses of functional divergence in amino acid sequences between electric and non-electric fishes, as implemented in FunDi [47], show significant evidence for divergent sites in complex I of South American electric fishes (electronic supplementary material, figure S4). Similarly, for the African dataset, the majority (78.6%) of divergent sites were found to be in complex I subunits. Within complex I subunits, the largest number of sites were found in the proton-pumping channels: ND2, ND4 and ND5 (electronic supplementary material, figure S4). We were unable to obtain functional divergence scores for the African COI and ND4L residues, most likely owing to short internal branch lengths.

(b) Strong positive selection associated with the origins of electrogenesis

We conducted branch-site analyses of the South American and African concatenated mitochondrial gene datasets to investigate whether the origins of electrogenesis led to

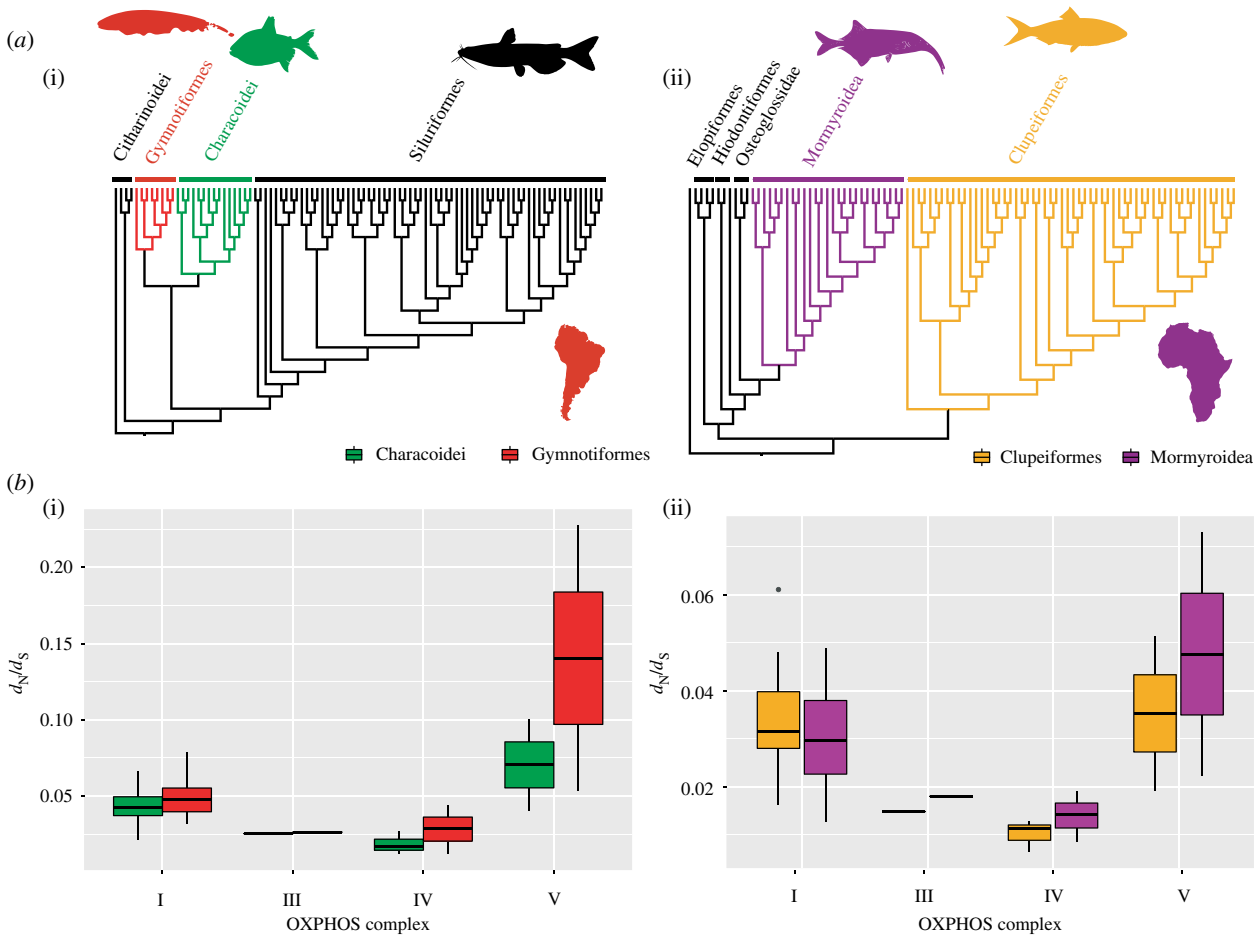

Figure 1. Convergent patterns of evolution of mitochondrial-encoded subunits of OXPHOS. (*a*) Phylogenies for taxa in the two datasets used to investigate OXPHOS evolution of weakly electric fishes from South America (i) made up of Characiformes, Gymnotiformes and Siluriformes species, and Africa (ii) consisting of Osteoglossiformes, Elopiformes, Hiodontiformes and Clupeiformes species. (*b*) Box plot of global d_N/d_S rates, using model M0, grouped by complex for Gymnotiformes compared to their non-electrogenic closest relatives, Characoidei (i), and comparison of d_N/d_S rates between the African electric fishes, Mormyroidea, to their non-electric relatives, Clupeiformes (ii). (Online version in colour.)

changes in selection in OXPHOS genes. Our results from the concatenated mitochondrial dataset show significant evidence for strong positive selection in the lineages leading to the ancestors of both Gymnotiformes ($d_N/d_S = 61.65$) and Mormyroidea ($d_N/d_S = 11.95$). Ten sites in the South American dataset and 12 sites in the African dataset were found to belong to the positively selected site class with high posterior probability (above 0.8) (figure 2*b*). Of these positively selected sites, the majority were found to be located in complex I: 70% of the South American dataset sites and 58% of the African dataset sites (electronic supplementary material, table S8). These positively selected sites are exclusively found in ND2, ND4 and ND5 subunits of complex I in both datasets (figure 2*a*).

In ND2, residue 326 was found to be under positive selection in both the South American and African datasets, with a convergent, non-conservative Leu to Thr substitution in both electrogenic ancestors (figure 2*a*), with posterior probabilities of 0.93 and 0.98 in the South American and African datasets, respectively (figure 2*b*). Some of the positively selected sites in ND5 are found along the amphipathic helix HL (figure 2*a*), which is known to be important in interactions among the ND2, ND4 and ND5 subunits, and important for complex I stability [22,50]. Separate branch-site analyses of each gene individually showed similar trends, but with lower statistical support as expected (electronic supplementary material, tables S10 and S11).

(c) Heterogeneity of rates of evolution in electric fishes

Figure 3 shows the d_N/d_S estimates and proportion of sites in the highest site class of each OXPHOS gene for electrogenic and non-electric clades. In both electrogenic clades, d_N/d_S values show higher variability, compared to more homogeneous values for non-electric fishes. In addition, for genes with site classes of higher d_N/d_S values, the electrogenic clades have higher proportions of sites in these site classes. For example, the three genes with the highest third site class d_N/d_S in Gymnotiformes have 10–40% of sites in the third site class, while the three genes with the highest third site class d_N/d_S in Characoidei have less than 10% of sites in the third site class (figure 3). Interestingly, in the South American dataset, a higher proportion of sites are also observed in electric fishes in CYTB even though the d_N/d_S estimate is lower than non-electric fishes. For complex I, we found that the d_N/d_S estimates for subunits involved in proton translocation, rather than the coupling mechanism, tended to be more elevated in Gymnotiformes (electronic supplementary material, figure S5). This pattern was not observed in the African dataset.

4. Discussion

In this study, we used codon-based likelihood models in a comparative mitogenomic approach to investigate the

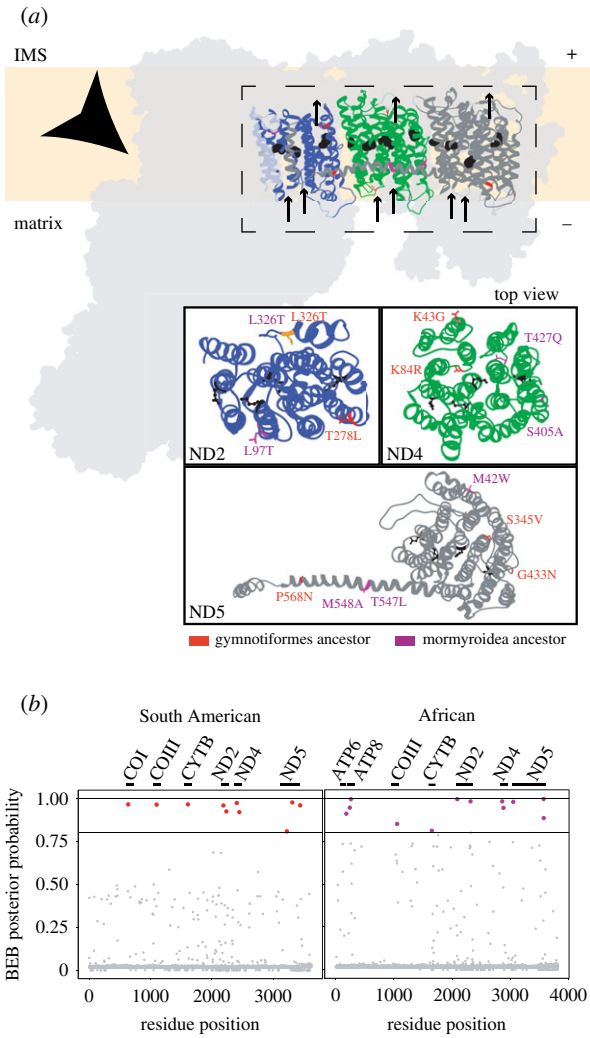

Figure 2. Positively selected residues along the branch leading up to the ancestor of electrogenic taxa. (*a*) Crystal structure of complex I showing only subunits ND2, ND4 and ND5 in ribbons, with functional residues that mitigate proton translocation in black, and positively selected residues in Gymnotiformes ancestor in red and Mormyroidea ancestor positively selected residues in purple. Orange residue (L326T) in ND2 subunit shows the convergent substitution in both datasets. Arrows indicate the proton translocation path proposed by Fiedorczuk *et al*. [22]. Insets show a top view of the subunits highlighting positions of positively selected residues, along with the substitution along the branch leading up to the most common ancestor of electrogenic fishes. Residue nomenclature follows the reference crystal structure, *O. aries*, numbering. Arrowhead shows the entry area of the quinone channel. (*b*) Posterior probability of each residue in the concatenated dataset belonging to the positive selection site class, as estimated by the BEB analyses. Horizontal lines highlight the boundaries (0.8–1.0) used to identify the positively selected sites along the ancestral branch in the South American (left) and African (right) datasets. (Online version in colour.)

impact of the evolution of electrosensory systems on the metabolic machinery of fishes. We investigated two groups of fishes (South American gymnotiforms and African mormyroids) in which electrosensory systems evolved independently, and used whole mitochondrial genome data to investigate the molecular evolution of OXPHOS complexes. Our analyses show that although OXPHOS genes generally evolve under strong purifying selection, there is statistically significant evidence of positive selection associated with the two independent origins of electrogenesis. Key positively selected residues were located in complex I, and may be associated with proton translocation. We also find evidence for a significantly elevated d_N/d_S within both electrogenic clades of South American and African fishes, most strikingly within genes comprising OXPHOS complex V. Here, we discuss our results in the context of the evolution of electrogenesis in electric fishes, and the increased metabolic burden associated with this sensory system.

(a) Positive selection associated with evolutionary origins of bioelectrogenesis

We found significant evidence for positive selection associated with the origins of bioelectrogenesis in the two independently evolved lineages of South American and African electric fishes, and identified key positively-selected residues in complex I. This suggests that complex I plays an important role in mediating the adaptation to increased metabolic needs associated with the evolution of bioelectrogenesis. Complex I is crucial to OXPHOS; it is the largest contributor to the proton gradient in the IMS [51,52]. It forms the first step in OXPHOS, and is known to be the rate-limiting step in neural oxygen consumption [53]. Reactions associated with complex I have been found to largely determine OXPHOS efficiency in nervous tissue such as the brain [54]. In addition, complex I dysfunction is the most common cause of human metabolic disorders [55–57]. The positively selected sites were mostly detected in ND2, ND4 and ND5 subunits of complex I, the Mrp Na^+/H^+ antiporter-like subunits known to translocate protons across the IMM [58]. The fact that no positively selected sites were detected in the subunits surrounding the quinone-binding pocket (ND1, ND4L, and ND3) further suggests that the coupling mechanism between the peripheral N-module catalytic arm and the hydrophobic membrane arm is probably not involved in the adaptation to bioelectrogenesis. We also find striking evidence in the ND2 subunit of complex I of a convergent substitution (L326T) in both ancestors of Gymnotiformes (South American) and Mormyroidea (African) electric fishes. Analysis of crystal structures of complex I suggests that the side chain of this residue orients inwards towards the proposed proton translocation path [22], thus the substitution of a polar residue may affect proton translocation efficiency. Some positively selected residues in ND5 are located along the amphipathic α-helix HL that bridges across ND2, ND4 and ND5 [59] pointing towards complex I accessory subunits [22]. This is in line with previous reports of positive selection in this region of ND5 [60], suggesting a role for these residues in interactions between ND5 and other subunits. Overall, in our dataset, it would appear that selection associated with the evolution of bioelectrogenesis may have affected cellular OXPHOS machinery associated with proton translocation, in the two groups of independently evolved electric fishes.

(b) Molecular evolution of oxidative phosphorylation subunits in electrogenic fishes

In addition to the positive selection of OXPHOS genes at the origins of bioelectrogenesis, our study also found significantly elevated d_N/d_S ratios in electric fishes compared to non-electrogenic relatives. This elevation in rate is relatively consistent across sites, and does not involve large d_N/d_S increases at some positions or dramatic differences in the

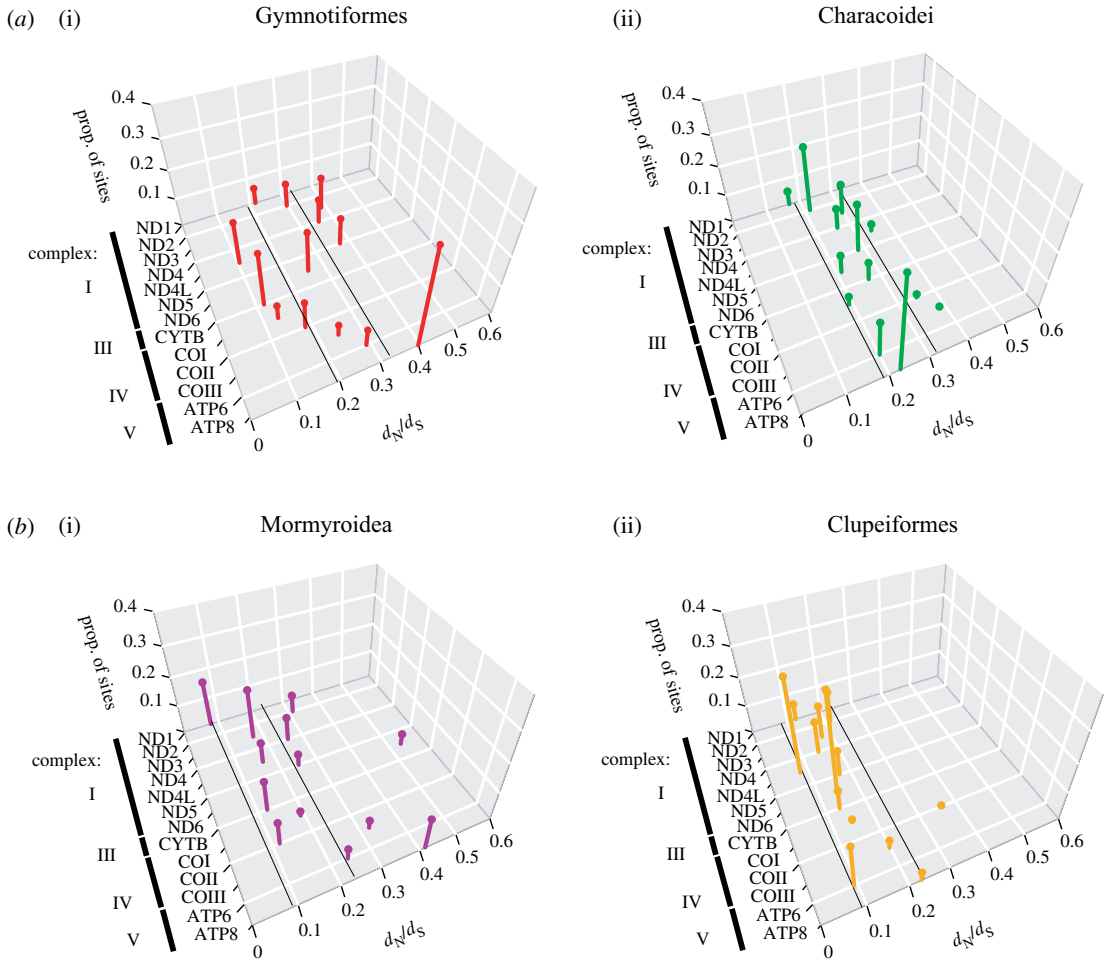

Figure 3. Heterogeneity of rates of gene evolution between electric and non-electric fishes. Three-dimensional plots of d_N/d_S estimates, proportion of sites across genes. (*a*) South American dataset, with electric fishes (i) and non-electric Characoidei (ii). (*b*) African dataset analyses, with the plot in (i) showing the estimates for the electric fishes, while those in (ii) are for non-electric relatives. Black lines show the mean-centred bounds of standard deviation of d_N/d_S estimates across OXPHOS genes of Characoidei (*a*(ii)) and Clupeiformes (*b*(ii)) as a reference for evolutionary rate heterogeneity. (Online version in colour.)

overall pattern of variance of d_N/d_S across sites (electronic supplementary material, figure S6). Assuming no change in synonymous substitution rates, this implies increased rates of non-synonymous substitutions in OXPHOS genes in electric fishes. An evenly distributed elevation of non-synonymous substitution rates across sites suggests higher selection pressures for increased function, but perhaps not the evolution of novel aspects of structure/function. The increase in evolutionary rate is particularly marked for complex V, and to a lesser extent complex IV. Complex V mediates a critical step in ATP synthesis, using the proton gradient across the mitochondrial membrane to couple proton translocation to ATP synthesis via a rotary action in the F_1 region [26,61,62]. The mitochondrial subunits (ATP6 and ATP8) are both located within the F_0 portion of the ATP synthase, and are involved in the protonation of the c-ring that further mediates the rotary action of the F_1 region [26,63]. Amino acid variation in this region may therefore affect this rotary action and proton translocation efficiency. Complex IV, on the other hand, mediates the transfer of electrons from the *Cyt c* carrier molecule to oxygen molecules, the final electron acceptor in the electron transport chain and suppression of complex IV has been found to result in a reduction in respiration rates [25], suggesting that variation in this complex may affect the rate of oxygen consumption.

In other studies, elevated d_N/d_S patterns have been found in animals that show reduced metabolic burden, and in these cases, elevated d_N/d_S has been attributed to relaxed selection rather than adaptive evolution [64–67]. Codon-based likelihood methods alone cannot distinguish between these two interpretations. However, given that: (i) evidence of positive selection is detected at the origin of electrogenesis in both datasets, with similar OXPHOS genes affected, (ii) physiological studies indicate that electric signals consume a large proportion of the energy reserve of electric fishes [5,18,68,69], and (iii) behavioural studies demonstrate that fishes modulate electric signals in response to metabolic stress [69–72], we believe that an adaptive explanation for elevated d_N/d_S rates is more reasonable than the competing hypothesis of relaxed purifying selection. We note that differences in evolutionary rates can be influenced by other biological factors, such as differences in population size [73–75] or generation time [76,77]. Further studies promise to elucidate the suite of factors affecting differences in these molecular rates.

Interestingly, it has been established that EO discharges produced by different species of gymnotiforms and mormyroids have varying energetic costs, depending on characteristics such as signal amplitude, frequency and overall type (wave versus pulse type signals) [71,78]. For example, electroreceptor afferents of wave-type fishes have a high firing rate, which could add to the overall energetic requirements of electrosensory systems [18,79]. Higher evolutionary rates in complexes IV and V within electric fishes may therefore reflect the evolutionary effects of variation in energy demands of the

different signal types, and the oxygen requirements to meet the energetic demands of these signals. In addition to the metabolic differences attributable to aspects of the electric signal, there is also notable variation in behavioural adaptations associated with electrosensing [2], as well as habitat differences, particularly in terms of conductivity [80]. Fishes that live in low conductivity water generally have longer and thinner tails than those living in water with intermediate conductivity, and the thickness correlates to the number of longitudinal layers of electrocytes [81]. All of these factors contribute to increased variation in metabolic demand across electric fish clades, which would be consistent not only with overall elevated evolutionary rates of OXPHOS genes in electric fishes, but might also explain the more heterogeneous evolutionary patterns of selective constraint across genes in the electrogenic (versus non-electrogenic) groups that we found in our analyses.

(c) Evolution of oxidative phosphorylation genes and linking genotype to phenotype in animals

There has been increasing interest in investigating mitochondrial function using comparative sequence approaches in order to identify shifts in selection in animals where differences in physiology or habitat are likely to have resulted in varied metabolic demands [82–84]. Generally, it has been found that mitochondrial OXPHOS genes are under strong constraint, with d_N/d_S well below 1 [82,84]. Against this background of purifying selection, however, there is mounting evidence of episodic positive selection associated with major changes in physiology or ecology. Significant evidence for positive selection in both mitochondrial and nuclear OXPHOS genes has been found at the origins of bats, associated with huge changes in physiology and ecology, including the evolution of powered flight [85]. Analyses of snake mitogenomes found striking evidence for positive selection at the origins of snakes, concurrent with extensive physiological changes in morphology and metabolism [86]. Unlike other studies which mainly found positive selection in OXPHOS genes of complexes I and V, in snakes, it appears that COI (complex IV) and CYTB (complex III) were most affected. A study of killer whale mitogenomes also found evidence for adaptive substitutions in CYTB from Antarctic populations [87]. More recently, a study found strong evidence for positive selection associated with the origins of soft-shelled and pig-nosed turtles, both of which evolved cutaneous respiration along with the loss of hard shells [83]. In that study, most of the positively selected sites in both turtle groups were found in the ND2 subunit (complex I), suggesting the potential role of ND2 in the adaptation to the metabolic changes associated with the evolution of cutaneous respiration in turtles. These findings are most similar to our results, in which we also found the majority of positively selected sites in ND2. However, in addition, we found a convergently evolved substitution (L326T) towards a polar residue in both electric fish groups, located in the proposed proton translocation pathway. This convergently evolved substitution suggests a possible adaptive role in the enhancement of the proton translocation across the IMM.

The increased availability of mitogenome sequence data, combined with advances in molecular evolutionary sequence analysis, has made it possible to investigate and better understand the biochemical mechanisms of metabolic adaptations that may be associated with major shifts in physiology. Our study, combined with those of others, suggest an emerging picture for mitochondrial genes in which metabolic adaptations are mainly centred in genes that are essential and rate limiting in the generation of the proton gradient (complex I), with increased rates in the machinery associated with coupling the proton gradient to ATP synthesis (complex V). Future studies should consider the evolutionary rates of the nuclear-encoded subunits of OXPHOS complexes, as they have shown elevated evolutionary rates matching those of mitochondrial origin [88,89]. Our study highlights the power and use of comparative analyses of selection to reveal the molecular basis of adaptations that appear to be important in the evolution of novel sensory systems.

Data accessibility. Access to all data used in this paper, including Genbank accession numbers, are included in the electronic supplemental material.

Authors' contributions. A.A.E., N.R.L. and B.S.W.C. designed the study. A.A.E. conducted computational and statistical analyses, and wrote the first draft of the manuscript. A.A.E., N.R.L. and B.S.W.C. interpreted the results, and revised the manuscript. All authors read and approved the final manuscript.

Competing interests. We declare we have no competing interests.

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