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NEWS AND VIEWS

PERSPECTIVE

Evolution of salinity tolerance from transcriptome to physiological system

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The relationship between microevolution and macroevolution is a topic of fundamental importance in evolutionary biology. The increasing accessibility of genomic tools is making the hunt for genes that underlie evolutionary divergence more tractable and, when combined with physiological approaches, provides exceptional power to elucidate the causal mechanisms of the relationship. In this issue of *Molecular Ecology*, Whitehead *et al.* (2013) employ this strategy to show that common physiological and genomic mechanisms lead to divergence in salinity tolerance across micro- and macroevolutionary timescales. They compare two killifish species from the genus *Fundulus*, *F. majalis*, which inhabits primarily marine and brackish environments and represents the ancestral state of the genus, and *F. heteroclitus*, which has derived an osmotic niche that expands into freshwater. Corresponding to the differences in osmotic niche, the species differ strikingly in how the structure of the ion-transporting epithelium and the transcriptome of the gills respond to osmotic challenge. These inter-specific differences were similar to but more pronounced than the differences associated with the more subtle intra-specific variation in osmotic niche within each species. It appears that a progression of the same functional adjustments first allowed expansion of the osmotic niche of *F. heteroclitus* into freshwater and then further expanded the niche of select *F. heteroclitus* populations towards more dilute freshwater environments. The work of Whitehead *et al.* therefore emphasizes how the mechanisms of adaptive divergence between populations can be expanded over time to produce the more complex differences that can exist between species.

Keywords: environmental adaptation, euryhalinity, evolutionary physiology, fish, osmoregulation, transcriptomics

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The fundamental niche of a species is presumably a major determinant of its distribution, yet the long-standing question of how microevolutionary processes associated with environmental adaptation culminate in the often large differences between species in both phenotype and distribution continues to this day (Reznick & Ricklefs 2009). An important component of this question is whether the underlying mechanisms of macroevolution are the same as those of microevolution. Comparative studies of physiological and transcriptomic responses to environmental challenges can provide key insights into this issue, because they can pinpoint the key genes, biochemical pathways and organ systems that are involved in adaptation. Indeed, mechanistic approaches are becoming increasingly common to understand the causal links between levels of biological organization that translate genotypic variation into phenotypic innovation and diversification (Dalziel *et al.* 2009; Storz & Wheat 2010).

Nearly all of the complex chemical reactions that define life are intimately dependent upon water and the solutes it contains. Fish that differ in osmotic niche therefore provide a useful tool to understand the mechanisms of environmental adaptation. Seawater and freshwater are starkly contrasting extremes of the osmotic environments that fish typically face. Seawater is approximately 3 times saltier than the body fluids, so fish have a tendency to lose water and gain salts by passive diffusion. The opposite is true in freshwater, which can be approximately 100 times less salty than body fluids and tend to cause fish to gain water and lose salts. These passive movements of water and ions would be catastrophic if left unabated, causing cells to shrivel up or swell and burst. The gills of fish are particularly important for counteracting these passive movements, by either absorbing ions in dilute environments or excreting ions in saline environments (Evans 2008). Although most fish are restricted to either seawater or freshwater, 2–3% of species possess the extreme physiological plasticity in the gills and other tissues that is needed to move between these contrasting environments (Schultz & McCormick 2013).

Previous intra-specific comparisons between populations with different osmotic niches have identified some of the putative physiological and genomic mechanisms of environmental salinity adaptation. Intra-specific variation in the capacity to remodel the cell structure, epithelial ion permeability and ion transporter expression of the gills has been associated with variation in osmotic niche breadth in multiple species (Scott *et al.* 2004; Brix & Grosell 2012; Whitehead *et al.* 2012). Associated with this intra-specific variation in salinity tolerance and gill remodelling are divergent transcriptomic responses to salinity change for genes involved in trans-epithelial ion transport, cell-volume regulation, cell stabilization and immediate early signal transduction (McCairns & Bernatchez 2010; DeFaveri *et al.* 2011; Whitehead *et al.* 2011, 2012).

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In this issue of *Molecular Ecology*, Whitehead *et al.* (2013) show that the physiological and genomic mechanisms of adaptive fine-tuning within a species also contribute to observed macroevolutionary differences between species that inhabit widely different osmotic niches. They do so by studying two species in the genus *Fundulus* that differ in their tolerance of freshwater. The euryhaline killifish *F. heteroclitus* (Fig. 1a) occupies a very broad osmotic niche, spanning the full range from seawater to freshwater. The striped killifish *F. majalis* occupies a more restricted osmotic niche in marine and brackish water environments. Fish were collected from three populations of each species from native coastal and estuarine habitats along the east coast of North America (Fig. 1b, c) and then maintained in artificial seawater in the laboratory. Fish from each population were then experimentally transferred to freshwater and their physiological and molecular responses were compared.

There was clear variation between species in the ability to tolerate freshwater, consistent with the differences in their natural osmotic niches. *F. majalis* lost control of ion and water balance (as reflected by decreases in plasma osmolality and ion concentrations) and consequently suffered high mortality in dilute freshwater, whereas *F. heteroclitus* suffered relatively little osmotic imbalance and no mortality. Associated with the inter-specific variation in freshwater tolerance were differences in the extent to which the gills of each species could remodel in response to salinity change. Scanning electron microscopy showed that *F. heteroclitus* could fully transform their gills to a freshwater phenotype, containing a rich abundance of ion-absorbing mitochondrion-rich cells (Fig. 2). In contrast, *F. majalis* was only capable of partially transforming its gills and suffered visible damage as a result of osmotic cell swelling. These striking inter-specific differences are similar, but more pronounced than the intra-specific differences between populations of *F. heteroclitus* that differ in freshwater tolerance (Scott *et al.* 2004; Whitehead *et al.* 2011, 2012).

There was pronounced divergence between species in the transcriptomic response of the gills to freshwater that was associated with the inter-specific differences in tolerance and physiology. The dominant pattern was that a

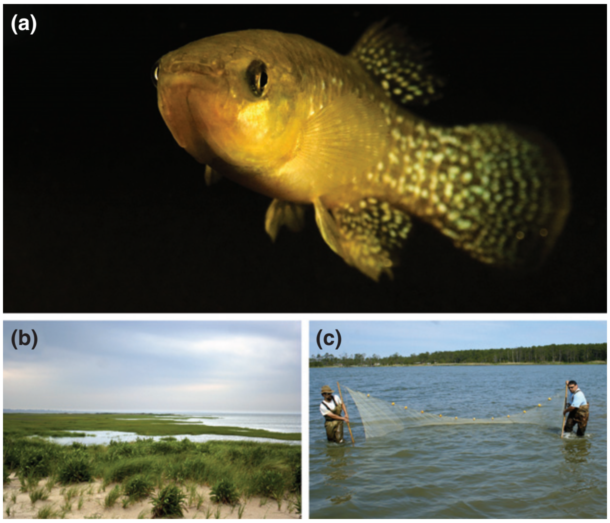

Fig. 1 (a) The euryhaline killifish or mummichog (*Fundulus heteroclitus*) has a broad osmotic niche that extends from marine to freshwater environments (photograph credit: Andrew Whitehead). The striped killifish (*F. majalis*), by contrast, is normally restricted to seawater and brackish water estuaries. (b) Fish from each species were caught from estuaries in Chesapeake Bay in Maryland (photograph credit: Fernando Galvez) and from coastal sites in Virginia and Georgia. (c) Authors Andrew Whitehead and Fernando Galvez at the field site in Chesapeake Bay (photograph credit: Shujun Zhang).

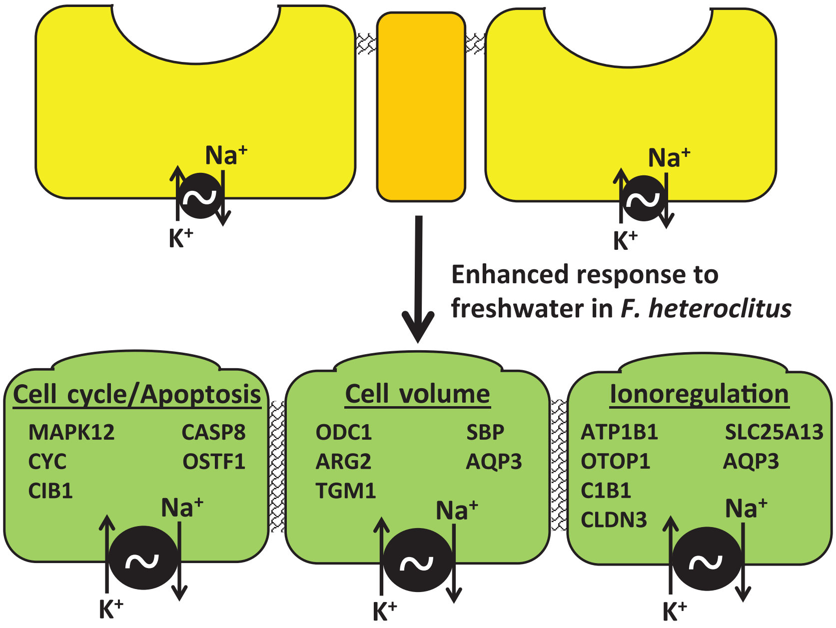

Fig. 2 The physiological plasticity important for entering freshwater is enhanced in *Fundulus heteroclitus* compared to *F. majalis*. The gills of *F. heteroclitus* exhibit a more complete morphological transition to a freshwater phenotype, with a loss of the seawater-type mitochondrion-rich cells (MRCs) that contain apical crypts (yellow) and a gain of the freshwater-type MRCs that have a convex appearance and a reduced ion permeability at the tight junctions between cells (green). Associated with the enhanced morphological transition to freshwater are transcriptomic differences in a number of genes involved in cell cycle regulation and apoptosis, cell-volume control and ionoregulation.

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large number of genes were differentially expressed after freshwater transfer by a much greater magnitude in *F. heteroclitus* than in *F. majalis* (Fig. 2). These include genes involved in active ion absorption (e.g. Na^+/K^+-ATPase β_1 subunit), epithelial permeability (e.g. claudin 3), cell-volume regulation (e.g. aquaporin 3) and osmotic signal transduction (e.g. p38 MAP kinase, osmotic stress transcription factor 1). Relatively few genes were differentially regulated by a greater magnitude in *F. majalis* than in *F. heteroclitus*. Included in this small category were genes involved in cell cycle regulation, which were down-regulated in *F. majalis*, perhaps as a response to cellular osmoregulatory failure. There was also a large set of genes whose response to freshwater was similar between species, and which probably contribute to the ancestral plasticity that is important for coping with dilute brackish water, but is insufficient for maintaining osmotic homoeostasis in freshwater.

Although a relatively large number of genes were regulated differently by osmotic challenge between species, it is likely that many are part of coregulated transcriptional networks (Whitehead *et al.* 2012). Some of the differences in transcript abundance may also arise as a consequence of the cellular restructuring in the gills. Presumably, there are some genes that are only expressed in the freshwater-type mitochondrion-rich cells (MRCs), and the transcripts of these genes should be more abundant in the gills of *F. heteroclitus* in freshwater because this species has more MRCs rather than higher gene expression *per se*. It is therefore possible that this complex array of adaptive transcriptomic differences is controlled by relatively few genetic loci. Determining whether this is the case would provide important insight into the mechanisms of osmotic adaptation and how they might contribute to ecological speciation and niche differentiation.

Regardless of the ultimate cause, it is clear that an enhanced capacity for physiological plasticity was derived in *F. heteroclitus* from a less plastic fundulid ancestor. Furthermore, the subset of genes whose expression responded differently to osmotic challenge between species was enriched for those genes that were found in a previous study to contribute to adaptive divergence in osmotic niche among populations of *F. heteroclitus* (Whitehead *et al.* 2011). It therefore appears that an increase in the capacity to remodel the gills, reflected at both the tissue and transcriptomic levels, first expanded the osmotic niche of *F. heteroclitus* into freshwater. Further enhancement of the same physiological and molecular plasticity was then employed to expand the niche of select *F. heteroclitus* populations into even more dilute freshwater environments.

The work of Whitehead *et al.* (2013) represents an important step forward in understanding how adaptive divergence between populations can lead to large differences in fundamental niche between species. Just as the themes and intricacies of a novel cannot be understood without appreciating the importance of phrases, paragraphs and chapters, the mechanistic basis of evolutionary change cannot be fully understood without appreciating the functional divergence of cells, tissues and physiological systems. The integrative approach of Whitehead *et al.* (2013) does just that, thus providing important insight into the mechanistic relationships between gene expression, biochemical pathways and organ system function that underlie evolutionary change.

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