Andrew Whitehead – Research

Overview & Research Paradigm

Broadly speaking, activities in the Whitehead lab revolve around Environmental Genomics research, as I seek to understand how genomes integrate cues from, respond to, and are shaped by the external environment. I examine genomic responses to stress that occur over physiological timescales (acclimation responses, toxicity responses) and over evolutionary timescales (adaptive responses). I exploit naturally evolved variation in stress resilience (among populations or species) to offer more nuanced insights into the mechanistic basis of organism function, and the evolutionary and landscape processes that partition that variation in space and time.

Many complementary approaches are integrated into my program, including genome-wide transcriptome profiling, population genetics, phylogenetics, physiology, toxicology, and evolutionary comparative approaches, to study the genomic basis and evolutionary history of response to environmental stress. Stressors of interest include those that are natural (temperature, salinity) and of human origin (pollutants). Importantly, anthropogenic stress in wild populations interacts with natural stressors in the real world, and a particular future focus of my research is to gain a mechanistic understanding of how exposure to pollutants can compromise organismal performance in the face of natural stressors such as temperature, salinity, hypoxia, and ocean acidification.

Model Organisms

Our current model organisms include killifish of the genus *Fundulus*. Why do we study Environmental Genomics using killifish? Fish are naturally exposed to a wide variety of environmental stressors by nature of their immersion in aquatic habitats. Killifish are typified by high physiological resilience. That is, they are physiologically plastic and capable of adjusting their phenotype to cope with environmental stress, yet species vary in their tolerance ranges. Different species occupy diverse habitats, and some of these habitats are more "stressful" or more variable than others. Populations and species of *Fundulus* have evolved tolerances to different environmental extremes, and therefore provide a wonderful comparative system in which to study the genomic basis of resilience or sensitivity to stress. Given all of the natural stressors that killifish are exposed to (temperature, salinity, hypoxia, pH) these fish are appropriate models for studying how pollution stress may interact with and compromise naturally evolved environmental stress responses. Interestingly, though killifish are generally resilient to natural stressors, they are sensitive to pollutants such as PCBs and dioxins compared to other fish species. I am a founding member of the *Fundulus* Genomics Consortium (Burnett et al. 2007), within which we have developed a well-resourced genomics toolkit for *F. heteroclitus*, and have started sequencing its genome.

F. heteroclitus is an important model for our current toxicology research for two reasons. First, populations living in highly polluted estuaries have evolved extreme tolerance to PCBs and dioxins, providing a naturally evolved system for studying mechanisms of dioxin toxicity and sensitivity. Second, killifish are the most abundant

vertebrates in Gulf of Mexico exposed marshes, and are currently serving as an important model for our research on the ecotoxicological impacts of the Deepwater Horizon oil spill. Future research could expand to include other ecotoxicological stressors of particular relevance to California, such as agricultural chemical runoff and decreasing pH from ocean acidification, for which appropriate models might include salmon, delta smelt, sea urchins, or intertidal snails. Importantly, the genomics revolution is enabling research where choice of model species dictates genomics research agendas rather than the prior availability of genome-scale resources.

Ongoing Projects

1. Genomic basis of parallel evolved pollution tolerance:

Multiple populations of *Fundulus heteroclitus* have independently evolved to tolerate extraordinarily high concentrations of PCB, PAH, and dioxin contaminants. Large populations live and thrive in EPA-designated Superfund Hazardous Waste sites, and can tolerate more than 1000X the concentration of PCBs that kills fish from other populations. This dramatic tolerant phenotype is heritable, has evolved over a very short time period, and has evolved independently at least four times. As such, this system provides an opportunity to explore compelling questions including "what is the genomic and physiological basis of naturally evolved variation in sensitivity to environmental pollution?" and "has repeated evolution of pollution tolerance drawn on mutations within the same or different genomic elements in parallel-evolved populations?".

We are taking three top-down approaches to addressing these questions. First, we are using QTL mapping of hybrid crosses between multiple tolerant and sensitive populations to determine how many loci are involved, and whether the same loci are implicated in different tolerant populations. Second, we are genotyping many wild individuals from multiple populations using thousands of markers for association studies to determine outlier loci. Third, we are using transcriptome profiling of PCB-challenged fish to determine what the individuals from tolerant populations are doing differently at the functional level to enable tolerance, compared to individuals from sensitive populations. This work is being done in collaboration with Dr. Diane Nacci (US EPA). To date, our research has shown that killifish populations have repeatedly adapted to extreme levels of dioxins and PCBs by evolving desensitization of the aryl-hydrocarbon receptor signaling pathway (Whitehead et al. 2010b; Whitehead et al. in review).

2. Toxic, compensatory, acclimation, and evolutionary responses of resident marsh fishes to the Deepwater Horizon oil spill:

My group has initiated an integrative research program in response to the Deepwater Horizon oil spill to assess the proximate and long-term effects on economically and ecologically important fishes resident in at-risk marshes along the Gulf coast. This work is being done in collaboration with Dr. Fernando Galvez (LSU). Soon after the oil release, we quickly mobilized and collected tissue samples and water samples from six field sites from Barataria Bay in the west to Mobile Bay in the east, to serve as pre-oil samples which are critically important for robust time-course analysis. Rarely are "before-event" samples available for ecotoxicology studies. Our objectives are 4-fold: 1. Document the time-course of exposure to the Gulf Killifish *Fundulus grandis* in Gulf-

exposed marshes using environmental chemistry and body burden analysis; 2. Use transcriptomics, protein biochemistry, and tissue morphology analyses to characterize the time-course of toxicity/stress response in situ in *F. grandis* in Gulf-exposed marshes; 3. Expose *F. grandis* embryos in the lab to field-collected waters to characterize the time-course of developmental effects, including transcriptomics to infer mechanisms of effect; 4. We have a large population sample (~150 fish) from a site "pre-oil". We plan to characterize their response to controlled hydrocarbon exposure, then return to populations during exposures, and post-exposure (at least one generation), to test for population-level longer-term acclimation and adaptation responses. This work is being partially supported by two grants awarded in 2010, and we are currently seeking additional funding to further support this research effort.

Field study data are currently under analysis, but our preliminary results are exciting. One of our six field sites was particularly hard-hit by contaminating oil, which we have documented through satellite imagery and analytical chemistry of water, sediment, and fish tissues. Within-site time-course analysis and between-site comparisons reveal a dramatic genome expression response in fish livers from the oil-impacted site. In previous studies we have identified genes that are transcriptionally-responsive to PCB exposure in killifish (Whitehead et al. 2010b; Whitehead et al. in review), and this set of PCB responsive genes is predictive of the transcriptomic divergence we observe in fish from our oil-exposed field site. Physiology data from my collaborator Fernando Galvez (LSU) shows extensive damage in the same fish in liver, gill, and head kidney tissues, which is tightly correlated with induction of CYP1A1 in all tissues. To my knowledge, these are some of the first "effects" data from the Deepwater Horizon oil spill, especially in resident vertebrates. We are linking exposure at multiple levels (satellite imagery and analytical chemistry) to effects at multiple levels (molecular and physiological), and preliminary results from our comparisons across space and time appear compelling. We expect a manuscript ready for submission within one month.

For future research, my group is particularly interested in how pre-exposure to oil compromises acclimation responses to salinity, hypoxia, and temperature: periodic and stochastic stressors that are common in the natural habitat of these fish. Relevant grants are in preparation.

3. Comparative evolutionary genomics of physiological plasticity:

Some species can change their phenotype to compensate for broad changes in environmental conditions, whereas other species have narrow tolerance ranges. Gene regulatory mechanisms are likely to underpin this physiological plasticity. We seek to define the transcriptomic basis of a universally important compensatory response (acclimation to osmotic stress) for several taxa that vary in their ability to tolerate stress based both on laboratory studies and on habitat distributions in nature. Evolutionary comparative analyses of variation in transcriptomic responses are being used to test for gene regulatory differences that account for species differences in physiological plasticity.

Specifically, functional genomic responses, integrated with physiological responses to osmotic challenges, are being compared within and among *Fundulus* species that vary in osmotic compensatory ability in the laboratory and in salinity habitat niche breadth in nature. We employ phylogenetic comparative analyses in order to distinguish

transcriptomic and physiological variances that appear evolutionarily neutral or highly conserved from those that appear functionally related to compensatory ability (physiological plasticity) and associated niche breadth. These species contrasts strategically test the evolutionary, ecological, and physiological significance of the maintenance or divergence of transcriptomic patterns. This research has resulted in several publications, including one in *PNAS* (Whitehead 2009, 2010; Whitehead et al. 2010a; Whitehead et al. in press).

Importantly, these studies are serving as a foundation for our ecotoxicology research, wherein we seek to understand how exposure to anthropogenic stressors can compromise responses to natural stressors. For example, many of the genes that are mis-regulated in Deepwater Horizon oil spill exposed fish (see research project 1 outlined above) are genes involved in the osmotic compensatory response. Gill tissues of oil exposed fish appear particularly damaged, so we hypothesize that oil exposure, in addition to direct toxicity, may compromise the ability of killifish to compensate for daily (tidal) and seasonal variation in their osmotic environment.

Future research

My group will continue to exploit the killifish model to mechanistically understand how anthropogenic stressors can interact with naturally-encountered stressors to compromise physiological resilience. Beyond this system, I have been keen to apply our integrative genomics approach to understand how climate change-driven ocean acidification may compromise growth and development in intertidal and estuarine organisms that also must contend with natural extremes in temperature, desiccation, hypoxia, and salinity. Importantly, there are regions of the Pacific coast that vary in exposure to deep-ocean upwelling. Regions exposed to upwelling (such as Monterey Bay) may harbor populations that have locally evolved tolerance to low pH, thereby providing natural comparative systems for discovering the genes and genomic programs important for resilience to low pH. Appropriate models may include sea urchins, mussels, or snails. Being at UC Davis would help facilitate this research direction, due to the proximity of appropriate habitats, and proximity of colleagues and potential collaborators already working on some of these systems (such as at Bodega Marine Laboratory). In addition, my Ph.D. research was centered on discovering the proximate and long-term effects of contaminating agricultural chemicals in California's Central Valley (Whitehead et al. 2003; Whitehead et al. 2004; Whitehead et al. 2005). These issues remain, and if I return to California I would come newly equipped with tools and paradigms of integrative genomics and physiology, which would enable a fresh approach to tackling complicated issues associated with estimating effects and impacts in native animals from the individual to population levels.

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