Flexibility and Constraint in the Evolution of Gene Expression and Behavior

Eva K. Fischer

Department of Biology, Colorado State University, Fort Collins, Colo., USA

Central to understanding phenotypic evolvability is an understanding of the extent to which the mechanisms underlying a given phenotype are flexible versus constrained. Are there many possible mechanistic solutions for a given adaptive problem, or are biological systems constrained such that solutions are limited? Evidence that shared neural circuitry and molecular substrates (e.g. neuropeptides, hormones, signaling molecules) mediate similar behaviors exists across vertebrates [Striedter, 2005; O'Connell and Hofmann, 2011], and some studies argue for even more phylogenetically widespread conservation of mechanisms [Arendt and Nübler-Jung, 1996; Strausfeld and Hirth, 2013]. Contrasting these patterns of widespread overlap is evidence that distinct molecular mechanisms may generate similar phenotypes in closely related species, populations of the same species, or even among individuals in the same population [Crawford and Oleksiak, 2007; Goaiard et al., 2009; Verster et al., 2014]. Evidence of distinct mechanisms mediating similar phenotypes suggests that multiple mechanistic solutions are possible and biological systems are indeed flexible. But what is the importance of mechanistic flexibility for the maintenance and evolution of biological systems?

Our understanding of how underlying mechanisms influence the evolvability of behavioral phenotypes remains limited. To shed light on this issue within the specific context of brains and behavior, I conducted three related studies that examined patterns of flexibility and constraint in the Trinidadian guppy, *Poecilia reticulata*. Guppies have become a model system in ecology and evolutionary biology due to their ability to rapidly adapt to novel environments. In Trinidad, guppies from high-predation sites have repeatedly and independently colonized low-predation environments, leading to parallel evolution in life history traits, morphology and behavior [reviewed in Magurran, 2005]. Moreover, we are able to tease apart genetic and environmental influences on adaptive phenotypes in the lab [Fischer et al., 2014]. Taking advantage of this unique system, my goal was to characterize forces facilitating and constraining behavioral evolution, and to explore interactions between developmental plasticity and evolutionary divergence in this context.

To explore patterns of flexibility and constraint at the level of behavior, I measured 21 behaviors across four assays and examined patterns of variation and covariation in behavior based on differences in evolutionary history with and lifetime ex-

posure to predators (fig. 1). Contrary to classical predictions that trait correlations are stable across time and space [Lande, 1979; Schluter, 1996; Merilä and Björklund, 2004], I showed that behavioral correlations observed in one population or environment did not predict correlations in another and that environmentally induced flexibility in behavioral correlations shaped evolutionary trajectories. Recognition that trait correlations are flexible, even within the lifetime of an individual, will inform both theoretical predictions and empirical studies of adaptation.

Given evidence for flexibility at the level of behavior, I next asked how such flexibility manifested in underlying transcriptional mechanisms. My experimental design allowed me to link baseline differences in gene expression to multiple behavioral phenotypes and to connect variation in gene expression to variation in behavior

The author won the 'Thomas Karger Award for Excellence in Evolutionary Neuroscience'. The award was presented at the 2015 J.B. Johnston Club meeting on October 16, 2015.

Fig. 1. In guppies, fish from distinct drainages represent unique evolutionary lineages in which parallel, adaptive phenotypes have evolved in high- and low-predation environments. **a** The experimental design allowed me to discern genetic influences of predation [contrast between high-predation (HP) and low-predation (LP) populations] from developmental influences [contrast between fish from the same population reared with (pred+) and without (pred-) predator cues]. By comparing guppies from distinct river drainages, these comparisons could be made for repeated evolutionary transitions. **b** I explored the evolutionary conse-

quences of patterns of flexibility and constraint at three levels. (1) I compared correlational patterns in suites of behavioral traits. Heat maps were used to highlight differences in correlation structures between groups. (2) I characterized the transcriptome at the level of individual genes and gene networks to understand whether alternative transcriptional states – at both the level of single genes and gene networks – are associated with similar behavioral outcomes. (3) I examined transcriptional signatures of adaptation to predator-free environments across repeated, independent evolutionary events.

within and among groups (fig. 1). I observed alternative transcriptional states predicting behavioral differences across rearing environments and genetic backgrounds, despite a similar range of behavioral variation across these groups. These observations provide evidence for mechanistic flexibility by demonstrating that multiple underlying mechanisms (i.e. transcriptional states) can give rise to similar phenotypes (i.e. behaviors). The transcriptional changes I identified may represent either gene expression shifts that drive adaptive phenotypic differentiation or compensatory changes in gene expression that maintain phenotypes in the face of en-

vironmental change. In either case, flexibility at the transcriptional level may increase evolvability by balancing the maintenance of favorable trait constellations with the generation of phenotypic diversity during adaptation to novel environments.

Finally, I took advantage of parallel phenotypic evolution in guppies to compare gene expression signatures associated with adaptation across independent evolutionary lineages (fig. 1). I found evidence for both shared and distinct transcriptional mechanisms associated with parallel phenotypic evolution. Together with results from the first and second study, the

findings of this third study suggest a potential role of for mechanistic flexibility in increasing evolvability; alternative mechanisms for similar patterns of trait divergence may maintain cryptic variation that can be released and selected upon when environmental conditions change [Gibson and Dworkin, 2004; Schlichting, 2008; McGuigan and Sgrò, 2009].

In concert these studies demonstrate striking flexibility across behavioral phenotypes, behavioral mechanisms and repeated evolutionary events. Moreover, they suggest three ways in which flexibility may influence evolvability: (1) by enabling changes in trait correlations and

thereby reducing pleiotropic constraints imposed by these correlations, (2) by allowing compensatory transcriptional changes that maintain phenotypes in the face of environmental change and (3) by allowing underlying cryptic transcriptional variation that may facilitate adaptation under novel environmental conditions. Although our understanding of how differences in underlying mechanisms influence behavioral evolution is still limited, I propose that mechanistic flexibility increases evolvability. Guppies are known

for rapid adaptation, and it remains to be seen whether the flexibility I observed is unique to rapidly adapting species or a fundamental principle governing how biological systems balance robustness and evolvability. Ultimately, a clearer description of mechanistic flexibility will promote our understanding of the forces limiting and potentiating adaptive evolution, and deepen our knowledge of how organisms respond to novel and changing environments.

Acknowledgements

I would like to thank the award selection committee, the organizers of the JB Johnston Club annual meeting, and Karger Publishers for the honor of receiving The Thomas Karger Award for Excellence in Evolutionary Neuroscience and the invitation to write this piece. Many thanks also to my doctoral advisor, Dr. Kim Hoke, and the members of the Hoke Lab at Colorado State University, without whom this work would not have been possible.

References

- Arendt D, Nübler-Jung K (1996): Common ground plans in early brain development in mice and flies. *Bioessays* 18:255–259.
- Crawford DL, Oleksiak MF (2007): The biological importance of measuring individual variation. *J Expt Biol* 210:1613–1621.
- Fischer EK, Harris RM, Hofmann HA, Hoke KL (2014): Predator exposure alters stress physiology in guppies across timescales. *Horm Behav* 65:165–172.
- Gibson G, Dworkin I (2004): Uncovering cryptic genetic variation. *Nature* 5:681–690.
- Goaillard J-M, Taylor AL, Schulz DJ, Marder E (2009): Functional consequences of animal-to-animal variation in circuit parameters. *Nat Neurosci* 12:1424–1430.
- Lande R (1979): Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution* 33:402–416.
- Magurran AE (2005): *Evolutionary Ecology of the Trinidadian Guppy*. New York, Oxford University Press.
- McGuigan K, Sgrò CM (2009): Evolutionary consequences of cryptic genetic variation. *Trends Ecol Evol* 24:305–311.
- Merilä J, Björklund M (2004): Phenotypic integration as a constraint and adaptation; in Pigliucci M, Preston K (eds): *Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes*. New York, Oxford University Press, pp 107–129.
- O'Connell LA, Hofmann HA (2011): The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *J Comp Neurol* 519:3599–3639.
- Schlichting CD (2008): Hidden reaction norms, cryptic genetic variation, and evolvability. *Ann NY Acad Sci* 1133:187–203.
- Schluter D (1996): Adaptive radiation along genetic lines of least resistance. *Evolution* 50:1766–1774.
- Strausfeld NJ, Hirth F (2013): Deep homology of arthropod central complex and vertebrate basal ganglia. *Science* 340:157–161.
- Striedter GF (2005): *Principles of Brain Evolution*. Sunderland, Sinauer.
- Verster AJ, Ramani AK, McKay SJ, Fraser AG (2014): Comparative RNAi screens in *C. elegans* and *C. briggsae* reveal the impact of developmental system drift on gene function. *PLoS Genet* 10:e1004077.