Costs and constraints in the evolution of phenotypic plasticity

Organisms respond to changes in their environment. This has long suggested to evolutionary biologists that this responsiveness, referred to as phenotypic plasticity when genetically encoded, may be largely adaptive[1]. The range of plasticity among traits and among organism, however, can be quite dramatic, an indication that the adaptive value of plasticity varies across environments.[2] What, then, are the conditions that promote or constrain the evolution of trait plasticity?

Theoretical models suggest that plasticity should evolve in heterogeneous environments in which the optimal phenotype varies with the environment, unless constrained by certain costs or limitations.[4,5] Costs can be associated with the maintenance and production of plastic responses or discordance between a phenotype and environment when an inappropriate phenotype is displayed for a particular environment (phenotype-environment mismatching). The evolution of plasticity may also be constrained by the reliability of the environmental information that organisms can use to predict or accurately respond to environmental changes (cue reliability).[6]

I hypothesize that costs associated with phenotype-environment mismatching are high and that they are tied inextricably to limitations associated with environmental information reliability. Without dependable environmental cues an organism cannot accurately display appropriate phenotypes for a given environment. I also suggest that phenotype-environment mismatching is far more likely to occur even with reliable cues if environmental heterogeneity does not occur with some regularity. Thus plastic responses to environmental heterogeneity can only evolve if 1) the changing selective pressures or stresses within those environments occur with some regularity and 2) there are sufficiently reliable environmental cues to indicate oncoming changes. The purpose of this study will be to test the role of these two variables in the evolution of phenotypic plasticity using experimental evolution and generalizing via mathematical modeling.

<u>Study System:</u> An experimental evolutionary study of plasticity requires a system that is evolvable under short time periods and possesses phenotypic variability in an easily distinguishable and measurable trait. By placing yeast (*Saccharomyces cerevisiae*) under selective pressure for faster settling rates in liquid media, Ratcliff et al. have evolved multicellular strains of yeast.[7] These multicellular clusters of yeast have been shown to vary significantly in size within and among populations. Additionally, cluster size in multicellular yeast populations appears to be evolutionarily flexible and is easily selected using settling speed as an indicator of size.[8] The relative simplicity of the system lends itself to mathematical modeling, allowing direct testing of model assumptions.

<u>Experimental Design:</u> I will subject strains of multicellular yeast to a dichotomous settling selection regime, alternately selecting individuals that remain suspended in a liquid medium longer ('floaters') and those that sink fastest ('sinkers'). Each culture will experience selective events seven times per week. Variations on this selection regime will be used to separate the relative effects of regularity in environmental change and cue reliability in plasticity evolution.

Cue Reliability: I will culture the yeast in a liquid yeast extract, peptone, and dextrose (YPD) medium. To moderate potentially confounding effects I will use fructose as a predictive cue, an alternative sugar that yeast readily respond to but that does not produce any significant extraneous effects. I will establish multiple treatment cultures in which I will vary the degree of reliability of the fructose to predict the type of future selection event. As a baseline for high reliability, fructose will be consistently added prior to every ‘sinker’ selection event. Other treatments will experience this fructose cue with increasing deviance from the strict fructose/sinker-selection association baseline. Three cue reliability experimental runs will be conducted examining interaction effects with a range of regularity in selection events (low, mid-range, high) based on the regularity baselines described below.

Regularity: I will set up multiple treatments that experience increasing degrees of irregularity in intervals between selection events. As a baseline for high regularity, selection events will occur at strict 24-hour intervals. Alternative treatments will experience selection events with decreasing regularity. The low regularity baseline treatment will experience seven selection events occurring randomly over a seven-day period. No cue will be added to control for any interaction effects.

Measuring Plasticity After 60 days of selection, cells will be imaged in a hemocytometer chamber using an Olympus IX-70 with a Scion CFW-1310C camera. Cluster size will be measured using ImageJ, which has previously been done in the Travisano lab[7,8]. Plastic responses within culture will be quantified by examining variance in cluster size within each culture with and without the presence of the fructose cue and at periods corresponding to settling selection in the high regularity experiment.

Predictions Larger cluster sizes permit multicellular yeast to sink faster thus providing them with an advantage during selection for ‘sinkers’. Smaller clusters, however, fare much better when there’s selection for ‘floaters’. Thus if there is an alternation between these two selection regimes, the optimal size will consistently change. There are three possible evolutionary responses that may result: divergence or the canalization of two separate size morphs that fluctuate in frequency, intermediate-sized morphs that don’t perform optimally under either regime but do okay in both, or plastic morphs that can facultatively switch responses to match changes in selection regime. Based on my hypothesis, *my predictions are that 1) higher reliability of a detectable environmental cue in predicting relevant environmental change will result in the evolution of greater plastic responses, 2) that this will also be true with increased regularity of environmental change without cue but to a lesser extent and that 3) high levels of both cue reliability and regularity will result in the greatest plastic responses.* Moreover, lack of selection event predictability both based on cue reliability and regularity will facilitate the evolution of a fixed intermediate size response.

Broader Impacts: It is my intent to use the knowledge gained from this project to engage with students who may not typically get to experience much more than textbook science. In this vein, I have begun working with Teaching SMART, an organization that engages elementary students in inner city schools with hands-on lessons on subjects in science and math. I am currently developing interactive lessons that introduce students to basic topics in evolution. Additionally, this next semester I will be working with and be mentoring an undergraduate student who will be working in our lab.

Literature Cited: [1] Fagen, R. 1987. *Evol Bio* 1: 263-271. [2] Van Buskirk, J. and U. Steiner. 2009. *J Evol Biol* 22: 852-860. [3]Berrigan, D. and S.M. Scheiner. *Phenotypic Plasticity* 82-86 (Oxford U. Press 2003). [4] Gabriel, W. *et al*. 2005. *Am Nat* 166:229-353. [5] Moran, N.A. 1992. *Am Nat* 139: 971-989. [6] Auld, J., A. Agrawal, and R. Relyea. 2010. *Proc R Soc B* 277: 503-511. [7] Ratcliff *et al.* 2012. *PNAS* 109: 1595-1600. [8] Rebolleda-Gomez, M. *et al.* 2012. *Artificial Life* 13: 99-104.