Evolution of phenotypic plasticity in a synthetic regulatory network.

Manjunatha Kogenaru[1], Philippe Nghe[1], Franck J. Poelwijk[1], Sander J. Tans[1]

***Short Abstract* — We study the evolution of a regulatory network in heterogeneous environments: a synthetic cascade controls the expression of enzymes providing a cost or a benefit for the bacteria. We measure the outcome of natural selection performed on mutant pools in different complex environments, combining cues and ressources which independently impact expression and fitness. Knowledge of genotype, phenotype and fitness allows us to analyse optimization of already existing regulatory phenotypes, as well as evolution of new regulatory functions by reversion the inducers. We identify evolutionary contraints and trade-offs imposed by the network topology and show how they can be overcome.**

***Keywords* — Darwinian evolution, phenotypic plasticity, trade-off, synthetic regulatory network, logic gate**

I. Introduction

Regulation is central to adaptation to complex environments [1]. Though, quantitative data on phenotype, fitness, environment and their relations are generally difficult to obtain and disentangle, which seriously limits mechanistic interpretation of the evolution of genetic regulatory networks in the context of phenotypic plasticity [2,3]. We propose here to explore the evolution of a synthetic regulatory network that allows to characterize and model quantitatively these different layers.

II. Experimental design

A synthetic plasmid transformed into *E.Coli* strains contains a cascade of two repressors, *tetR* and *lacI*, induced respectively by *dox* and i*ptg*. This cascade controls the expression of three downstream proteins : *LacZ*, reporter of the expression level, *sacB* reducing growth in *sucrose* media, and *cmR* facilitating growth in presence of chloramphenicol (*cm*). Mutants pools of the repressor proteins and promoter regions are grown in environments consisting 4 alternating media, which are combinations of inducers and *sucrose* or *cm*. Thus, a phenotype, defined by expression as a response to inducers level, can be favoured or not depending on the presence of *sucrose* or *cm* [4]. Sequencing and phenotypic measurements performed on specific mutants reveal the evolution at the level of expression patterns, network structure and binding constants.

[1]Biophysics group, AMOLF, Science Park 104 1098 XG Amsterdam, The Netherlands, E-mail: nghe@amolf.nl

III. Results

A. Optimization of a native function

A first selection experiment is performed on an environment favouring low expression in the presence of only *dox*, which corresponds to the native phenotype of the network. Natural selection based on our artificial cost-benefit system is effective and leads to an optimized phenotype by tuning the binding constants of the two repressors.

B. Evolution of new regulatory phenotypes

In a second experiment, selection is performed in an environment favoring low expression only in the absence of inducers. Analysis of the phenotype-fitness relation determines the constraints imposed by the native topology of the network, represented as a bounded domain in the fitness space. Strikingly, the mutants obtained after one round of selection mostly spread on the optimal Pareto front of this domain, structurally corresponding to a knock-out of one of the inducing functions. A fraction of the mutants is able to overcome this limit by inversing *tetR* response to inducer and without change in promoter regions, as indicated by sequencing. This new regulatory phenotype can itself be optimized in a second round of selection. Similar features are observed in another experiment in an environment putting pressure on *lacI* role in the network.

IV. Conclusion

We are able to determine bounded domains in the fitness space representing the network topological constraints and show experimentally that these domains determine the outcome of natural selection in different environments. Optimal fronts of these domains can be overcome by evolution of new regulatory functions of repressor proteins modifying the network topology.

References

[1] Levins R (1968) Evolution in changing envoironments: some theoretical explorations. Princeton: Princeton University Press

[2] McAdams HH, Srinivasan B, Arkin AP (2004) The evolution of genetic regulatory systems in bacteria. *Nat Rev Genet* **5**:169-178

[3] Poelwijk FJ, Kiviet DJ, Weinreich DM, Tans SJ (2007) Empirical fitness landscapes reveal accessible evolutionary paths. *Nature* **445**:383-386.

[4] Poelwijk FJ, de Vos MGJ, Tans SJ, Environmental tradeoffs predict the evolution of phenotypic plasticity, in preparation.