EvoEvo Deliverable 3.3

Paulien Hogeweg, Guillaume Beslon

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FP7-ICT FET Proactive EVLIT program
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Evolution of evolvability; Mechanisms and consequences

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1. Introduction

Evolvability is a basic property of living systems. Indeed living systems are defined by Joyce (2012) in "Bit by Bit: The Darwinian Basis of Life" as:

"*Biological systems are distinguishable from chemical systems because they contain components that have many potential alternative compositions but adopt a particular composition based on the history of the system. In this sense biological systems have a molecular memory (genotype), which is shaped by experience (selection) and maintained by self-reproduction*".

He makes two very important points:

o Evolution involves long term information integration as clearly emphasized in this quote. This aspect has long been neglected in evolutionary theory, where the emphasis has been on direct fitness benefits.

o It is far from trivial that a reproducing entity can indeed harbor enough variation that can be selected, i.e. can evolve. This is clear from his in vitro RNA based autonomously evolving system, in which only a tiny fraction of the RNA sequence can evolve: the rest should be conserved to sustain the system. This is in his Bit by Bit title, and the open challenge is how the ratio between "evolvable" bits and "borrowed" bits has (can be) shifted to more evolution.

These are indeed the challenges we try to tackle in the EvoEvo project.

In Deliverable 3.2 on the mechanisms and consequences of robustness we have shown that robustness and evolvability are intricately interrelated. Indeed our conclusion was: "*Robustness is an evolved property, one of the mechanisms for robustness is evolvability, and one of the consequences of robustness is evolvability*".

In this report we will first review a fundamental classical concept of non-evolvability, i.e. the error-threshold, which limits the information that can be accumulated through evolution. In our computational experiments allowing more degrees of freedom, we have gained new insights in how evolution tries to circumvent this problem. Next we review three perspectives from which questions about the degree of evolvability have been approached, and summarize our results in the light of these. Next we add a detailed discussion on the role of types of mutations, i.e mechanisms for the generation of variability, for innovation in well adapted (virus-like) individuals and populations. Finally we highlight some inroads to understanding the evolution of complexity, showing that more complex replicators can evolve despite strong selection pressures countering the complexity in the short run. In the conclusions we show how the obtained insights on evolvability are to be used in WP4 ("A computational EvoEvo framework") and WP5 ("EvoEvo applications") and lead up to our discussion of open-ended evolution in D3.4.

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2. A Classical concept of Non- Evolvability: The Error threshold and novel insights

Any discussion on evolvability has, of course to start with Darwin.

Darwin based his theory of evolution on an impressive body of observations on the variability of species and the inheritance of this variability. His profound insight was that natural selection of this inheritable variation could be the mechanism that led to the existing, well adapted, diverse biosphere.

Once this mechanisms was formulated, and especially its shortened form as "survival of the fittest" it seemed so obvious that many people (e.g. the philosopher Popper) deemed it not a theory but a tautology.

It is not a tautology, but, nevertheless true for life on earth. Indeed, not all systems following Darwinian mechanisms evolve: there are conditions for evolvability.

One of the first clear formulations on these conditions was the recognition of the "Error Threshold" (Eigen 1979). If mutation rate is too high relative to selection (i.e. fitness difference or reproductive advantage) the fittest species is *NOT* selected. Mathematically this is simple expressed by the condition $1 - \mu > 1/\sigma$ where μ is the mutation rate and σ the fitness. This insight has an important corollary, called the information threshold. Because the relevant mutation rate per replicator should scale with the size of its genome, i.e. the information its contains, the error threshold translates into a limitation of the amount of information which can be selected, given a certain, per bit, mutation rate. This leads to Eigen's paradox: "in order to accumulate more information, mutation rate should be lowered, but in order to lower mutation rate we need more information (i.e. repair mechanisms)".

The error threshold problem is not completely resolved, but very profound insights related to this problem have emerged in our studies:

- o "*Not all mutations are equal*": We have shown that deterioration by high mutation rates can be relieved by increasing mutation rate, iff these extra mutations are biased to duplication/deletions rather than point mutations (the error threshold was formulated with only point mutations in mind). We have shown this in two different contexts, namely in mutator strains with a 100 fold increase of mutation rate, as known from E. coli (e.g. in the LTEE experiments) (Rutten et al., 2016a, b), as well as in the context of transcription induced mutations in Yeast (Colizzi and Hogeweg, 2016c).
- o "*Multiple coding*": One way to increase information content without increasing effective mutation rate is multiple coding in which one bit (nucleotide, gene, etc) is used for more than one function. Such multiple coding does not have to be designed, but evolves automatically (Beslon et al., 2016a, b; de Boer and Hogeweg, 2012) possibly decoded by the mutation process itself (Colizzi and Hogeweg, 2014).
- o "*U-shaped mutational neighborhood*": More generally, evolution tends to minimize the error threshold problem by evolving a U shaped mutational neighborhood, which maximizes

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selection coefficient as well as neutrality (Cuypers and Hogeweg, 2012, Rutten et al., 2016a, b).

In conclusion: *mutation rate shapes the genomic coding structure in such a way that deleterious effects of mutations can be minimized*.

3. Three perspectives on coding structure and degree of evolvability

Since Evolution has been used as a strategy for problem solving and optimization questions on the degree of evolvability have (re)surfaced. In this context evolvability is seen as whether and how evolution can fast reach a certain target (solve a certain problem).

From the beginning certain "desirables and obstacles" where recognized, and mostly formulated in terms of the shape of the fitness landscape. Thus a "mount Fuji" or a "royal road" fitness landscape, where there is a smooth continuously ascending route to the top were deemed the 'best'. In other words when the evolutionary process does not have to pass a fitness valley. Or in biological terminology if there is no "negative epistasis", or in computational terminology, when the problem is not "deceptive". Also, an intuitive notion is that evolutionary search will be most effective when the search space is as small as possible.

The shape of the fitness landscape is determined through the genome structure and the genotype to phenotype mapping, together referred to in the following as the coding structure.

In order to study and possibly quantify the question of evolvability to a certain target, three different perspective have been taken:

- o Given coding structure observed in biology, how does it impact on evolvability;
- o Given a problem how can it best be coded to allow efficient solution through evolution?
- o Given an evolutionary processes (starting from a certain coding structure) how does the coding evolve, and how does this affect evolvability. It is this last perspective that we primarily pursue in the EvoEvo project.

We will discuss important insights from these perspectives below.

3.1. Biological coding structure and evolvability

RNA sequence to (secondary) structure mapping has been extensively studied as a prototype of biologically realistic genotype-phenotype mapping because of its computability, as well as its biological relevance. This work has lead to the conclusion "RNA is an ideal evolvable molecule" (Schuster et al., 1994). Indeed, the RNA GP map is highly redundant, neutral networks percolate through genotype space, only relatively few mutations are needed to reach a predefined structure, and once a structure is found neutral drift brings an ever increasing set of alternative structures in the mutational neighborhood (Huynen 1996). It later turned out that these properties also hold for other biological genotype-phenotype maps (Wagner, 2005, 2008).

As discussed in D3.2 evolution on such landscapes lead to more robustness (neutrality) and more evolvability. In other words the landscape is rugged, but high dimensionality and the neutrality

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ensures there is a smooth path "to the top" (Huynen et al., 1996). Circumventing crossing the valley may require a long detour, but as calculated by van Nimwegen and Crutchfield (2000) the average time such a detour takes is smaller than that of crossing a valley for moderate mutation rates.

3.2. Coding for problem solving

The insights derived from studying biological coding structure are entirely opposite to the intuition for evolvability on which theory and practice has been based: the landscape is rugged, and high dimensional, instead of smooth and low dimensional. However, and unfortunately it is not a trivial task to design a landscape with these properties for a particular problem.

Nevertheless a number of recommendations for coding for problem solving can be made, some of which have been discussed in D3.1 on variability, as variability is of course a prerequisite for evolvability.

Here we list some heuristics for improving coding for problem solving, which have emerged from our work.

- o Embed populations in space, with competition only between neighbors. This helps to conserve variability in the population.
- o Use sparse fitness evaluation, i.e. determine fitness only on a subset of the target rather than complete fitness evaluation. At any time an easier problem is to be solved. Over time the evolutionary process integrates the information and solves all the problems. This strategy improves the efficacy of evolutionary search. This is even more so when the subset of problems co-evolves with the solutions, in such a way that they gain fitness by not being solved. This way the evolutionary process remains focused on the "what has to be done" (Pagie and Hogeweg, 1997; Pagie and Mitchell, 2002; Mitchel et al., 2006; de Boer and Hogeweg, 2007).
- o Allow flexible genome size, using other mutational operators than the classical point mutations and crossover. Not only in biological examples, but also in a function approximation tasks it has been shown that genome expansion early in evolution, improves attaining high fitness late in evolution (de Boer and Hogeweg, 2010), help coping with difficult tasks such as noisy targets (Batut et al., 2016a, b), help regulating mutation effects (Rutten et al., 2016a, b) and enable the finding of new paths on fitness landscapes (Beslon et al., 2016a, b).
- o Define mutation rates per position, rather than per replicator. This allows useful genome inflation, but simultaneously prevents unnecessary genome inflation (Fischer et al., 2014). Indeed continuous genome inflation often plagues solution found by genetic programming, the most used formalism with flexible genome size used for applications.

3.3. Evolution of coding structure: evolution of evolvability

Evolution of coding structure is a core topic in the EvoEvo project. Coding structure impacts on both robustness and evolvability, and these two concepts are therefore intertwined in the research we do, and the discussion cannot be easily separated. Therefore many results on evolution of

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evolvability have been mentioned in Deliverable 3.2. Here we will summarize them distinguishing between population based and individual based evolvability.

3.3.1. Evolution of Population based evolvability

In the perspectives discussed above, evolvability refers to the potential to reach some target, or solution to a problem, over evolutionary time, and how much time it will take to reach the solution. In other words this refers to the evolvability of the population, and operates over (long) evolutionary time.

We have seen that population based evolvability increases with neutrality, i.e. the fraction of mutated offspring with the same fitness, because this leads to larger variability in the population and more exploration. How effective this is depends on the more global structure of the landscape, i.e. the percolation and intermingling of different neutral networks (or phenotypes), the RNA type of landscape mentioned above being "ideal".

We have seen that:

- o Evolution leads to a 'U shaped' mutational neighborhood, i.e. relatively high neutrality and strong selection, which can be skewed to either side, dependent on circumstances (Cuypers and Hogeweg, 2012; Rutten et al., 2016a, b).
- o Note, however, that high apparent neutrality can be caused by a decrease of the number of mutations, e.g. by decreasing genome size: neutrality by non-mutation obviously does not increase exploration. However by conserving fit individuals it may increase the chance of rare fitness improving mutations to occur.
- o Evolved regulation increases population evolvability to match novel conditions (Cuypers et al., 2016; van Dijk and Hogeweg, 2016)
- o Different mutational operators influence evolvability differently, where LCR and duplication deletions are particularly effective owing to their multiplicative effect on genome size (Fischer et al., 2014).
- o Evolution tunes the ratio of different mutational operators, e.g., indirectly by evolution of genome size, operon structure and intergenic regions (Rutten et al., 2016a, b) and therewith increases both robustness and evolvability.
- o Evolvability, in the sense of faster fitness improvement of some individuals of the population and be 'bought' at the prize of lower average fitness of the population (i.e. by the increased mutation rate and the skewing the U shape to deleterious mutations) (Rutten et al., 2016a, b).

3.3.2. Evolution of Individual based evolvability

Relative to population based evolvability, individual based evolvability is not based on population variability, and directly refers to the mutational neighborhood of an individual. Interesting results on individual evolvability are:

- o After repeatedly experiencing alternative environments, mutational neighborhood evolves to become skewed such that beneficial mutations are overrepresented. (Crombach and Hogeweg, 2008; Cuypers et al., 2016).

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- o This individual based evolvability does not hinder population-based evolvability as neutrality is not diminished.
- o The bias to beneficial mutations is mediated by the evolved genome structure and/or by the evolved genotype to phenotype mapping, in particular the gene regulatory network.
- o Individual evolvability can lead to an average fitness of the ancestor lineage similar to the fitness obtained from a regulatory response to the environmental shift, and is easier to evolve than regulation.

4. Innovation in well adapted populations

As we discussed it in D3.2, evolvability and robustness are tightly interdependent concepts and should not be considered as antagonistic properties of an evolving system. Indeed, in some conditions robustness may enhance evolvability by increasing population variability (when the U-shaped mutational neighborhood is skewed to high neutrality). On the other hand, when robustness is based on a U-shape skewed to deleterious mutations, it may highly constrain evolvability. This has received an indirect illustration in a modeling experiment realized on digital viral strains with the aevol model. Indeed, these strains are highly constrained by robustness pressure that considerably limits their evolutionary possibilities and quickly leads them to get stuck in local optimum.

In order to understand how such strains find their escape path to start evolving again (i.e. increase their evolvability again after a long period of stasis) we used well-adapted populations having evolved for 200,000 generations in a constant environment. These populations were cloned and let evolved for 25,000 further generations. By selecting those that escaped their previous optimum to reach a higher peak (~20% of the populations), we were able to understand the path to innovation. The outcomes of this study are (Beslon et al., 2016a, b):

- o Innovation occurs in bursts of mutations (virtually all population that show fitness improvement gained more 80% of their fitness in less than 25% of the generations of the experiment).
- o Some populations are more prone to innovation than others – although we were not able to find the origins of these differences.
- o Importantly, the origin of innovation is NOT linked to moves on the neutral network, NOR to valley crossing (both being the two proposed mechanisms proposed in the literature). On the opposite, all innovations were triggered by a increase in evolvability[1].
- o This increase of evolvability can be due to the occurrence of deleterious events (linking evolvability to valley crossing) but in a large majority of the populations, it was due linked to events modifying genome structure, either locally (InDels) or globally (LCR). This leads to the important conclusion that, in these populations, innovation was NOT triggered by individuals moving on a fixed fitness landscape but rather by an evolution of the fitness landscape itself, in other words, an EvoEvo process. Indeed, this evolution of the fitness

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[1] In this study evolvability is considered as the probability of a given individual to improve its fitness. We developed an experimental measure that enables us to quantify evolvability along the lineage of a given individual (Beslon et al., 2016a, b).

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landscape opens the possibility of new mutations that were not possible previously since their locus were just not present in the genome.

To conclude, this experiment has revealed a new mechanism for the evolution of evolvability: *Evolution of the fitness landscape thereby adding new escape paths to an organism stuck on a local optimum*.

5. Beyond adaptability: evolution of complexity

The discussion above has equated evolvability with adaptability, which is indeed its most frequently used meaning. However, beyond adaptability, we observe the staggering complexity and diversity that is the result of Darwinian evolution, mutation and selection. Evolution of complexity is not obviously the result of adaptation, or even the increase of adaptability. For example the information threshold discussed above appears to be an obstacle. Indeed, to explain the evolution of complexity remains the great challenge of evolutionary biology in general and EvoEvo in particular.

A number of intriguing hints have emerged, with in their core long-term information integration in evolutionary processes. It is worth noting that long-term benefits have been a long-standing taboo in evolutionary biology. This had/has a good reason, i.e. to avoid just-so stories. However our constructive, non-supervised modeling approaches have shown that long-term information integration does emerge from short-term mutation selection processes, often leading to counter-intuitive results.

In the first place we have seen that increased genome size despite the resulting increased mutational load, may evolve and lead to:

- o Higher fitness in the long run (Cuypers and Hogeweg, 2012). Indeed this increase in early evolution is a generic property seen in all our experiments, as well as in phylogenetic reconstructions. Although subsequent evolution leads to gene loss, some apparently non-essential complexity remains after e.g. whole genome duplications, which is called "irremediable complexity" (Cuypers and Hogeweg, 2014).
- o Decreased mutational deterioration in the long run, while large genome size is maintained (Rutten et al., 2016a, b; Colizzi and Hogeweg, 2016c). This is mediated by a shift in mutational operators towards LCR and InDels which can be an automatic effect of larger genome size, and/or evolves through genome structuring.

Moreover, novel higher levels of selection emerge in spatially embedded eco-evolutionary systems through spatial pattern formation. Such higher levels of selection are essential for countering long term evolutionary deterioration that can result from the basic short-term selection process. A preeminent example is the decrease in replicase activity (strength of catalysis) in the RNA world because replicating someone decreases the fitness of the replicator, and leads to the emergence of parasites in such systems. This is true for any cooperative system. Emerging higher levels of selection prevent extinction (e.g. Takeuchi and Hogeweg, 2009; Colizzi and Hogeweg, 2016a, b). Preventing extinction is of course a first prerequisite for further evolution! Moreover the evolution of these higher levels represents by itself evolution of complexity. Here we highlight how these higher levels of selection also mediate the evolution of more complexity in the replicators themselves.

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o Evolution of DNA in the RNA world, one of the major transitions in evolution has been the separation of information storage (the genome) and information usage (interacting RNA's and proteins). We have shown that in multilevel systems this division of labor evolves despite the longer replication time it involves, because it mitigates the above mentioned selection pressure to lower catalysis, as this selection pressure is not acting on the inactive DNA. The system evolves such that information flow is from DNA to RNA – and so high catalysis is maintained. Thus the novel level of selection does not only prevent extinction, it leads to complexification of the lower level entities that in its turn mitigates the deteriorating selection pressure.

o Emergence of larger genomes despite severe selection for smaller ones. We have seen that the rich evolutionary potential of the StringMol molecule can only come to fruition when premature extinction through parasitism is prevented through spatial pattern formation (Hickinbotham and Hogeweg, 2016). In the model there is a strong selection pressure to minimizing genome size, as replication time is proportional to genome size. Indeed in early phases of evolution replicators become shorter (and parasites very short). Intriguingly late in evolution we begin to see very long replicators to gain a foothold. Moreover the replicators evolve very intricate 'smart' ways to avoid debilitating parasitism by themselves, e.g. by decreasing replication rates of parasites, independent of their length, or even by converting templates to primers, i.e. using parasites to replicate themselves, rather than the templates. Further analysis of these results is in progress, but so far this research is a powerful proof of principle of opening new avenues of evolution by an interplay of self-organization and evolution.

A common theme in the examples of complexification is the prevention of long-term evolutionary deterioration that result from short-term selection pressures. Moreover they highlight that simple evolutionary processes, through long term information integration can prevent this deterioration despite its cost in short term fitness, and the role of novel levels of selection in this process.

Thus we conclude that *short-term selection pressures leading to long-term evolutionary deterioration lead in the long run to the evolution of complexity; multilevel evolution plays a central role, enabling long-term information integration.*

6. Conclusion

As we showed it in D3.2, evolvability and robustness should not be opposed. Consequently, insights on the latter provide insights on the former and *vice versa* and, contrary to what was anticipated, tasks 3.2 (study of robustness) and 3.3 (study of evolvability) were strongly intertwined. Indeed, the *in silico* experiments presented in this deliverable and the one presented in D3.2 were often the same. Yet, these experiments provided important insights on the mechanisms that enable evolving systems to keep evolving – i.e. to maintain or increase their evolvability – under various conditions. Some of these mechanisms will be used in the artificial systems developed in WPs 4 and 5. In particular, we identified the following principles to be used in artificial evolution:

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- o Variable genome size and structure enables evolving systems to adjust their evolutionary dynamics to the environmental conditions. Variable genome size also enables initial genome inflation that has been shown to be highly valuable on the long run.
- o Genomes of variable size can be self-regulated by using a per-base mutation rate.
- o LCR and InDels provide alternative evolutionary paths and help regulating the mutational neighborhood by linking it to non-coding sequences and genome structure (when point mutations and crossover only "see" the coding sequences)
- o Spatial or temporal structure (e.g., evolving on a grid or evolving in cyclic environments) enables stabilization of the population structure, preventing population collapse due to parasites and leading to information integration on the long-term.
- o Individuals can escape local optimums by evolving their fitness landscape, hence opening alternative paths toward higher peaks.
- o In many situations, evolution of evolvability is linked to evolution of complexity. Indeed, the emergence of new structures adds new levels of selections that interact with the other – "lower" – ones thereby opening new evolutionary directions.

We started this deliverable by acknowledging that robustness and evolvability are intertwined concepts. We also know that robustness is directly linked to variability. The last item above closes the loop by suggesting that evolvability has also strong links with open-endedness and evolutionary transitions.

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