EvoEvo Project
FP7-ICT FET Proactive EVLIT program
Project reference: 610427

Deliverable 3.1
Variability: mechanisms and consequences
Version 1.2

EvoEvo Deliverable 3.1

Variability: mechanisms and consequences

Due date:	M24
Person in charge:	Paulien Hogeweg
Partner in charge:	UU
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Deliverable description:	Evolution of variability; Mechanisms and consequences: A report describing how variability is indirectly selected in the model and its consequences on evolution.

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1.1	Corrections and validation	03/11/15	G. Beslon (INRIA)
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1. Introduction

The mechanisms of biological evolution, as proposed by Darwin, are variability and selection. Whereas Darwin envisioned small phenotypic changes, and subsequent evolutionary theory (both within biology and computer science) has largely focused on point mutations, cross-overs and a simple genotype phenotype mapping, we are now starting to realize that the rich repertoire of genomic changes, as well as a complex (and evolved) genotype to phenotype mapping are major ingredients of the generative power of evolution. Indeed to unravel these mechanisms of generating variation at the genome and at the phenotypic level and their evolutionary consequences is one of the central goals of EVOEVO. Moreover we have come to realize that, apart from these intra-organismic sources of variability, it is essential to consider the variability of the environment in which the organisms evolve as well. In this report we will discuss our results on how variability is indirectly selected in the model and its consequences on evolution, from the viewpoint of (1) the mutational mechanisms (mutational operators) leading to genome variability, (2) the complex relation between genotypic and phenotypic variability in the population (3) consequences of environmental variability on genotypic and phenotypic variability. In our computational experiments these three aspects are, however, intertwined.

The computational experiments were performed in our main evolutionary platforms, i.e. (1) Aevol, which implements a detailed genome structure, and a fixed genotype to phenotype mapping, (2) PoS which has a course grained genotype structure but allows for the evolution of the genotype to phenotype mapping, (3) the Virtual Cell model which in addition to an evolvable genotype to phenotype mapping an simple metabolism evolves, so that not only evolutionary adaptation but also regulatory adaptation is possible and (4) the most general evolutionary models, the Virtual Microbe (an extension of the Virtual Cell model) and the integrated EvoEvo model, which can incorporate more extensive transcriptional and metabolic regulation (Rocabert et al., 2015).

It is important to use these different platforms to get insight in the underlying mechanism of the emerging evolutionary phenomena, their generality and the hidden constraints of various model assumptions.

Major general insights are highlighted in italics and indicated by bullet points.

2. Mutational Mechanisms

Point mutations are traditionally seen as the major source of genomic variation, whereas crossover in sexual reproduction is thought of as a mechanism to recombine point mutations occurring in the population. This is true in biology as well as in evolutionary computation. Recent analysis of whole genome sequences of bacteria has shown, however, that the frequency of accepted Horizontal Gene Transfers (HGT) as well as gene deletion are much more frequent than point mutations (Puigo et al., 2014). Moreover the Long-Term Evolutionary Experiment (LTEE) of *E. coli* (Raeside2014) and other shorter term evolutionary experiments have shown that large-scale chromosomal rearrangement (LCR) occur frequently.

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- *Our research has highlighted that these sources of variation have different roles and are not additive.*

2.1. Large scale chromosomal rearrangements (LCR)

LCR, i.e. duplication and deletion of variable length stretches of the genome change the size of the genome. Frequency of mutations in the genome is proportional to genome size. Thus LCRs indirectly influence effective mutation rates and therefore automatically tunes the generation of variability.

A very generic property of evolution, which we observe in all our computational evolutionary experiments, irrespective of the variability of the environment, and levels of the organization incorporated, is:

- *Initially, in an unadapted state, and after environmental change, when population have low fitness, LCR lead to genome expansion. This is followed by long term streamlining. Much fitness increase is achieved during streamlining, i.e. by deletions (Cuypers and Hogeweg 2012, 2014; Batut et al., 2015).*

Note that this model prediction is sustained by phylogenetic reconstructions of various groups, (e.g. (Richards et al., 2014) that also recognizes this as a generic pattern of evolution).

Experiments in a constant environment, using the Aevol platform, with its detailed representation of genome structure, have revealed two other fundamental insights about LCR, i.e.

- *Assuming that not only frequency, but also size of LCR scales with genome size[1], LCR generates limited genome size even without selection (Fischer et al., 2014).*
- *LCR by itself is almost as efficient in generating high fitness as LCR, point mutation and deletions together, whereas only point mutations are much less efficient (Knibbe et al., in prep).*

This is true even when LCR occur at random positions. However, LCR are often mediated by transposons and Insertion sequences, whose location is the result of long term evolution.

In our computational experiments in a variable environment, using the PoS platform we have shown that:

- *Long-term evolution can organize genomes such that LCR allow enhanced evolvability, i.e. very fast adaptation to environmental change (Crombach and Hogeweg 2007, van Dijk unpublished results).*

In *E.coli*, the parallel loss of the ribose operon in all strains of the LTEE was indeed shown to be the result of the transposition of the adjacent IS150 element, followed by non random LCR (Cooper et al 2001).

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[1] This property is sustained by empirical data. Indeed, in eukaryotic genomes LCR are observed which length is longer than all prokaryotic genomes.

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We conclude that LCR is a powerful first order mechanism of evolution of evolution. Through LCR and the resulting changes of the genome size, effective mutation rates are automatically tuned to adaptive and neutral phases in the evolutionary process. Moreover LCR and the resulting changes in genome structure can bias the occurrence of specific mutations and therewith render the mutational process more effective.

2.2. Mutation rates, and genome organization

As mentioned above, genome size tunes the effective mutation rates of all mutational operators. On the other hand, the predetermined mutation rate parameters determine the size and the organization of the evolved genome. By simply changing these parameters in Aevol we have been able to general a "virus like" or a "E.coli-like" 'wild type' (WT) genome can be generated in long term evolution (MS9 and MS10). High mutation rates and large populations result in small virus-like genomes with overlapping genes and little non-coding DNA. These viruses are often transcribed in a single RNA with a very active promoter (a feature that is often observed in viruses). With a hundred fold lower point mutation and InDel rate but only a tenfold lower LCR rate, E.coli-like genomes are generated. They are larger, have more intergenic regions and mostly non-overlapping genes. The transcription structure is much more sparse than that observed for virus-like genomes but polycistronic structures (aka operons) are frequently observed as is the case in prokaryoric genomes.

Some of the LTEE strains evolved a mutator genotype, which has a hundred fold higher mutation rates. Contrary to the theoretical predictions, the fitness of these strains is not affected. Inspired by these results the effect of a hundred fold increased point mutation rate was studied in *in silico* evolved E.coli- like WT (Rutten et al., 2016; Rutten et al., in prep). Counter-intuitively, increase in the mutation rate leads to increase in genome size, thereby increasing effective, per genome, mutation rate even more. In other words, the same point mutation rate that from a random initial condition led to a small virus-like WT genome, applied to the much larger E.coli-like WT genome has an opposite effect increasing its size even further. Nevertheless, after a transient fitness loss, fitness remains about the same, i.e. the larger mutation rates do not impair the fitness of these larger genomes. In contrast, increasing mutation rates of the virus-like WT decreases genome size as well as fitness (Beslon, unpublished results).

- *Impact of increased point mutation rate depends on a prior genome structure (and rates of other mutational operators).*

Closer inspection of the in silico E. coli-like "mutator" strain shows that the genome size increase is located in the intergenic regions, while the size of coding genes is decreased. Therefore the increased genome size primarily enhances overall mutation rates, and a flexible genome structure. This is in nice agreement with the LTEE experiments which also show increased genome flexibility of the mutator strains (Raeside et al., 2014).

As high mutation rates 'push the population off target', and therewith increases directional selection, these results are in fact on par with the genome expansion after environmental change mentioned above. More general:

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- *Genome expansion, and the resulting higher mutation rates is a response to the pressure to adapt. This pressure to adapt can arise by environmental variability or due to imposed changes in the mutation rate (Batut et al., 2016; Batut et al., in prep; Rutten et al., 2016; Rutten et al., in prep).*

2.3. Horizontal Gene Transfer (HGT)

Horizontal gene transfer (HGT) is a very frequent mutational operator in bacteria. Recently (Puigo et al., 2014) estimated that accepted HGT events are an order of magnitude more frequent than gene deletion rate, which in turn is an order of magnitude more frequent than the per gene point mutation rate. In the LTEE HGT was deliberately excluded in order to study long-term clonal evolutionary processes in E. coli. However, *E. coli* itself evolved under influence of HGT. Therefore to study the long term evolution yielding the natural occurring bacterial genomes, the study of evolutionary consequences of HGT is needed.

HGT is often referred to as "sex" (i.e., recombination) in bacteria. However, more than is the case for sexual recombination, HGT rates of different genes can be very different. Therefore HGT enhances multilevel selection: i.e. selection of horizontally transferred genes, and selection of cells can act more or less independently, i.e. can act in consort as well as in conflict.

In a model of toxin and resistance genes in a spatial ecosystem, in which HGT rates per gene can evolve (van Dijk and Hogeweg, 2015, 2016), we have shown that:

- *Differential HGT rates evolve in closed ecosystems: toxin genes evolve high mobility whereas resistance genes evolve low mobility.*
- *Through differential gene mobility high population based diversity (variability) is generated and maintained. Deep phylogenetic differences are maintained despite adaptive sweeps.*
- *Like in bacteria, genomes evolve to consist of a core genome, shared by all members of a population, and an accessory genome that is very variable, and is 'used' for short-term adaptation.*
- *Differential gene mobility allows genes that are only seldom beneficial to remain in the population.*

HGT appeared to be an essential mutational operator in the Virtual Microbe evolutionary model for evolving complex WT cells in a variable environment (MS9-11). In identical but highly variable circumstances highly divergent evolution unfolded. Moreover, when the so evolved WT populations were transferred into constant environmental conditions, continuous evolutionary change and divergent evolution was observed in the cases that HGT was operational, but evolutionary stagnation occurred without HGT (Cuypers and Hogeweg, 2015).

In contrast in the integrated EVOEVO model of Rocabert et al (2015), in which de novo gene discovery is rampant, HGT is not needed for evolving complex metabolic organisms and ecosystems. Thus it appears that de novo gene discovery plays a similar role as HGT from distant sources. Similarly, in Aevol, analysis of de novo gene formation in the absence of HGT has shown that other large-scale events (i.e. LCR) can efficiently replace HGT in generating "novelty" in evolving genomes. This confirms the importance of large-scale events of all sorts in evolution.

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3. Genotypic and Phenotypic Population Variability

It is well known that sufficient genotypic and phenotypic variability is essential for effective evolution.

- *Spatial embedding and local interaction enhance population variability, and improve evolutionary outcomes.*

All our modelling platforms now allow spatial embedding and local interactions.

There is a complex relationship between genotypic variability and phenotypic variability and mutation rate/mutational operators.

The incongruities of genotype to phenotype variability are mediated by the genotype to phenotype mapping (GP map), which in natural populations (and in many of our computational frameworks) is an evolved property. Indeed the evolution of the GP map is a major mechanism for evolution of evolution, including evolution of robustness and evolution of evolvability. Therefore most results on the evolution of the GP map will be discussed in Deliverable 3.2 and 3.3.

Here we only highlight those aspects related to the incongruities of genotypic and phenotypic variability.

Indeed, one genotype can generate different phenotypes (plasticity) because of multiple attractors of the regulatory system that defines the GP map. Moreover, epigenetic inheritance can maintain this diversity over generations. Using R-aevol, a variant of aevol in which the GP map can evolve we have shown that:

- *Such epigenetic inheritance can speed-up evolution (Vadée-Le-Brun et al., 2015).*
- *The complexity of the GP map is influence by both the complexity of the environment and by the variability at the genome level (Vadée-Le-Brun et al., 2016)*

The GP map defines the fitness landscape. Traditionally steep (high selection) and flat (high neutrality) landscapes are distinguished. Flat landscapes lead to high genotypic variability and relatively low phenotypic variability and steep landscapes to relatively low genotypic and phenotypic variability which scales with mutation rate (for moderate mutation rates).

Many biological landscapes are characterized by percolating neutral paths, and evolution at moderate mutation rates lead to regions of higher neutrality, i.e. higher phenotypic variability. Here we list some new insights obtained in the variety of GP maps which evolve under different circumstances.

- *A "U-shaped" fitness landscape evolves in the virtual cell model, combining the 'best of both worlds: neutrality and high selection, i.e. many almost-neutral mutational neighbours and many (almost) lethal neighbours whereas slightly deleterious mutations seldom occur[2] (Cuypers and Hogeweg 2012).*

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[2] Such a U shaped GP map has been observed for viruses (Sanjuan et al 2004) and in yeast (Wochl et al 2001).

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- *At very high mutation rates population of interacting RNA replicators evolves to a steep region of the fitness landscape (or G-P map) where minimal variations from a master genotype map to large phenotypic differences, which acquire new functional roles in that they either help the replication of the master sequence, or they hinder the replication of its competitors (Colizzi and Hogeweg 2014).*
- *At lower mutation rates, in the same RNA world model, speciation, i.e. high genotypic and high phenotypic and functional differentiation evolves; the GP map of the various species varies from steep to very flat (Takeuchi and Hogeweg 2008).*

Moreover, long-term evolution in a variable environment can shape the GP map such that the phenotypic effect of random mutation is biased toward beneficial effects after predictable environment change. This will be discussed in Deliverable 3.3 (Evolvability, mechanisms and consequences).

An important conclusion for EvoEvo is that variability is a very complex trait. When considering population variability:

- *Multiple measurements are relevant; both genotypic and phenotypic variability should be distinguished, and should be expressed not only terms of frequency distributions of different genotypes/phenotypes, but also in terms of phylogenetic depth, mutational distance and functional differentiation.*

4. Environmental variability

Most natural populations experience a changing environment at multiple timescales. To cope with these changes population can adopt at least three different modes of adaptation: regulatory adaptation, evolutionary adaptation and polymorphism. Regulation generates phenotypic variability (i.e. plasticity), without genotypic variability, whereas evolutionary adaptation accomplishes phenotypic variability by genotypic variability. In polymorphism, the population is split into subpopulations that ecologically interact such that the ecological system is able to cope with environmental variations. These three modes are mostly studied in isolation.

Using the Virtual Cell model we studied the interrelation by employing different timescales of environmental variability in wild-type populations that evolved to maintain homeostasis in a resource environment that changes orders of magnitude on short timescales. In other words the WT populations evolved efficient regulating relative to one type of environmental change. The main conclusion is:

- *The ability to regulate enhances the ability to evolve. In other words environmental induced phenotypic variably enhances the potential of genotypic adaptation to a range of different environments (Cuypers et al., 2015).*

The LTEE experiments have evolved *E. coli* now for more than 60.000 generations in an environment that changes in a very predictable manner, whereas the WT *E. coli* populations evolved in a very variable environment. LTEE strains have become more fit in this predictable

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environment, and several parallel genotypic changes have occurred in independent evolutionary experiments. The genotypic changes hint at reduced regulation.

Using different model at our disposal (mostly EvoEvo integrated model and virtual microbe) we are studying this general protocol: Bringing WT strains evolved in variable environments in a constant or predictably varying environments. Following discussions with experimentalists in Grenoble, two levels of adaptation have been studied: (1) changes in global regulation and in particular on the relaxation of stringent response to the cyclically changing environment, (2) ecotypes differentiation as is observed in the S-L differentiation event (Plucain et al., 2014). Our results have shown that:

- *Environments varying in a cyclic way promote the evolution of structured ecosystems with cross-feeding ecotypes maintained by frequency-dependence (Rocabert et al., submitted).*

5. Conclusions

The results of our computation studies of biological evolution, and in particular of the mechanisms and consequences of variability at the level of genomes, populations, and in the environments have led to a deeper understanding of biological evolution. The most important points have been highlighted above.

Our results also provide some heuristics for evolutionary computation that are partly at odds with common practice. Below we summarize our main results from this perspective.

It is well known that sufficient population variability is needed for evolutionary optimization. Maintaining this variability is a well known problem in evolutionary computation. Our results have shown that variability can be self-regulated through EvoEvo mechanisms at least in the following conditions:

- *Embedding the population is space and, instead of global competition, let individuals only compete with their neighbours is a simple, computationally efficient and effective way of maintaining population variability and increasing evolutionary success. This has been tested – and confirmed – in the context of artificial evolution in WP4 (Hickinbotham and Hogeweg, 2016).*

In evolutionary computation the most often used genetic operators are point mutations and crossover. However,

- *Extending the mutational operators to include large chromosomal rearrangement (LCR), and horizontal gene transfer (HGT) extends the evolutionary potential.*

Determining optimal mutation rates is a much studied topic in evolutionary computation.

- *Allowing variable genome size and per position mutation rates, tunes mutation rates automatically, and leads to initial genome expansion followed by streamlining, ultimately leading to compactly coded solutions.*
- *Reorganization of genomic structures enables genomes to adapt to high mutational levels, thus reducing and, in some condition cancelling, the mutational burden.*

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An ecosystem of interacting evolving entities provides many degrees of freedom that can be exploited by evolution. It seems worthwhile to explore ecosystem-based problem solving:

- *An ecosystem of locally interacting problem solvers can automatically decompose problems, and increase the efficacy of evolutionary computation (de Boer and Hogeweg 2012; Rocabert et al., submitted).}*

Summarizing, we have used a variety a evolutionary modelling frameworks, each of adding degrees of freedom to evolving entities.

This is done by including different levels of biological detail to the models, e.g. flexible genome size, more mutational operators, complex and evolvable genotype phenotype mapping, embedding the evolving entities in space, and letting interactions evolve rather then predefining them. We have shown that doing so, up to now ill understood biological phenomena proved to be generic properties of evolution and that it opened up new promising avenues for evolutionary computation. Moreover, we have shown that by adding degrees of freedom evolution of evolution emerges.

Paradoxically adding degrees of freedom requires relatively complex models. Such models have often been avoided both in biology and in evolutionary computation because they are harder to make and to use. But, as Einstein apparently has said: "Models should be as simple as possible but nor more so".

6. References

B Batut, C Knibbe, G Marais and V Daubin (2014) Reductive genome evolution at both ends of the bacterial population size spectrum. *Nature Reviews Microbiology*, 12:841-850

B Batut, G Beslon and C Knibbe (2016) Unexpected genome inflation and streamlining in variable environments. *Proceedings of Jobim 2016, Lyon (France), June 2016*, pp. 320-322

B Batut, G Beslon and C Knibbe (2016) Genome inflation under variable environments. *In prep.*

ES Colizzi and P Hogeweg (2014) Evolution of functional diversification within quasispecies. *Genome biology and evolution*, 6(8):1990–2007

FK de Boer and P Hogeweg (2012) Co-evolution and ecosystem based problem solving. *Ecological Informatics*, 9:47-58

VS Cooper, D Schneider, M Blot and RE Lenski (2001) Mechanisms Causing Rapid and Parallel Losses of Ribose Catabolism in Evolving Populations of Escherichia coli B. *Journal of Bacteriology*, 183(9):2834-2841

A Crombach and P Hogeweg (2007) Chromosome rearrangements and the evolution of genome structuring and adaptability. *Molecular biology and evolution*, 24(5):1130-1139

TD Cuypers and P Hogeweg (2012) Virtual genomes in flux: an interplay of neutrality and adaptability explains genome expansion and streamlining. *Genome biology and evolution*, 4(3):212-229

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TD Cuypers and P Hogeweg (2014) A synergism between adaptive effects and evolvability drives whole genome duplication to fixation. *PLoS computational biology,* 10(4): e1003547

TD Cuypers and P Hogeweg (2015) Endless evolutionary paths to Virtual Microbes. *First EvoEvo Workshop, York (UK), July 2015, 9 p.*

TD Cuypers, JP Rutten and P Hogeweg (2015) Mutate or Regulate: evolutionary strategies along a continuum of ecological time scales (*in prep*)

B van Dijk and P Hogeweg (2015) Multi-level evolution, differential gene mobility, and the persistence of population diversity, *Proceedings of the European Conference on Artificial Life 2015, York (UK), July 2015,* pp. 9-11

B van Dijk and P. Hogeweg (2016) In silico gene-level evolution explains microbial population diversity through differential gene mobility. *Genome biology and evolution*, 8(1):176-188

S Fischer, S Bernard, G Beslon and C Knibbe (2014) A model for genome size evolution. *Bulletin of mathematical biology*, 76(9):2249-2291

S Hickinbotham and P Hogeweg (2016) Evolution towards extinction in replicase models: inevitable unless... *2nd EvoEvo Workshop, Amsterdam (NL), September 2016*, 5 p.

C Knibbe and DP Parsons (2014) What happened to my genes? Insights on gene family dynamics from digital genetics experiments. *Proceedings of ALIFE 14, New-York, July 2014*, pp. 33-40

C Knibbe, D Schneider and G Beslon (2016) evolution without (point) mutations. *In prep.*

J Plucain, T Hindré, M Le Gac, O Tenaillon, S Cruveiller, C Médigue, N Leiby, WR Harcombe, CJ Marx, RE Lenski and D Schneider (2014) Epistasis and allele specificity in the emergence of a stable polymorphism in Escherichia coli. *Science*, 343(6177):1366-1369

P Puigbo, A Lobkovsky, DM Kristensen, YI Wolf and EV Koonin (2014) Genomes in turmoil: quantification of genome dynamics in prokaryote supergenomes. *BMC Biology* 2014, 12:66

VP Richards, SR Palmer, PD Pavinski Bitar, X Qin, GM Weinstock, SK Highlander, CD Town, RA Burne and MJ Stanhope (2014) Phylogenomics and the Dynamic Genome Evolution of the Genus Streptococcus. *Genome Biology and Evolution*, 6(4):741-753

C Raeside, J Gaffé, DE Deatherage, O Tenaillon, AM Briska, RN Ptashkin, S Cruveiller, C Médigue, RE Lenski, JE Barrick and D Schneider. (2014) Large chromosomal rearrangements during a long-term evolution experiment with Escherichia coli. *MBio.* 5(5):e01377-14

C Rocabert, C Knibbe and G Beslon (2015) Towards an Integrated Evolutionary Model to Study Evolution of Evolution. *First EvoEvo Workshop, York (UK), July 2015, 15 p.*

C Rocabert, C Knibbe, J Consuegra, D Schneider and G Beslon (2016) Beware Batch Culture: Seasonality and Niche Construction Predicted to Favor Bacterial Adaptive Diversification. *Under review.*

JP Rutten, P Hogeweg and G Beslon (2016) Evolution of mutator populations in constant environments. *2ⁿᵈ EvoEvo Workshop, Amsterdam (NL), September 2016*, 5 p.

JP Rutten, P Hogeweg and G Beslon (2016) Adapting the engine to the fuel: hypermutator populations can escape the mutational burden by reorganizing their genome structure. *In prep.*

R Sanjuan, A Moya and SF Elena (2004) The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. *PNAS,* 101(22):8396-8401

N Takeuchi and P Hogeweg (2008) Evolution of complexity in RNA-like replicator systems. *Biology Direct*, 3:11

Y Vadée-Le-Brun, J Rouzaud-Cornabas and G Beslon (2015) Epigenetic inheritance speeds up evolution of artificial organisms. *Proceedings of the European Conference on Artificial Life 2015, York (UK), July 2015,* pp. 439–446

Y Vadée-Le-Brun, J Rouzaud-Cornabas and G Beslon (2016) In Silico Experimental Evolution suggests a complex intertwining of selection, robustness and drift in the evolution of genetic networks complexity. *Proceedings of the Artificial Life Conference 2016, Cancun, Mexico, July 2016*, pp. 180-187

DM Wloch, K Szafraniec, RH Borts and R Korona, (2001) Direct estimate of the mutation rate and the distribution of fitness effects in the yeast Saccharomyces cerevisiae. *Genetics*, 159(2): 441–452