# *Escherichia coli* adaptation to the gut environment, a constant fight for survival.

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*Escherichia coli* is an extremely versatile species with a high adaptation capacity to new and variable niches. It harbors an astonishing level of genetic and phenotypic diversity and can even assume the form of a deadly pathogen. But most members of the species live as commensals. Indeed *E. coli* is commonly sampled from the feces of many mammals and birds, and it is the predominant facultative anaerobic bacteria in the gastrointestinal tract. In humans it colonizes the gut within hours after birth [1] and is a typical stable inhabitant of our intestines, where it competes with other species of the microbiota. In the seventies Milkman analyzed hundreds of natural *E. coli* isolates and used multilocus enzyme electrophoresis to reveal an average genetic diversity of 0.23 in 5 loci [2], a value that later revealed to be a lower bound with the analysis of further loci [3]. Genetic variation in *E. coli*, like in many other bacterial species is likely the result of the well known evolutionary mechanisms: mutation, genetic drift, recombination, migration and natural selection. While mutation, the primary mechanism of generation of new alleles, genetic drift, the random sampling of alleles from one generation to next, recombination, the exchange of genes between different strains, and migration of clones between hosts are key processes which may account for some features of the observed *E. coli* population genetic structure and variation [4,5], natural selection is also thought to play a significant role. When a new advantageous mutation emerges in a given gene and increases in frequency, eventually fixing in the population (selective sweep), it leaves a signature on the pattern of nucleotide variation at nearby sites. In particular if selection is strongly favoring a beneficial mutation, the linked neutral alleles will hitchhike with it and genetic variation will be wiped out following such sweep [6]. Indeed the first suggestion of a global selective sweep in *E. coli* came from the analysis of levels of polymorphism of its gapA gene which exhibits a striking reduced variability in natural isolates of *E. coli*, amongst other observed patterns departing from the expectations of neutral evolution [7].

## *E. coli variation within a human host along time*

Even though *E. coli* is one of the most studied organisms, there is still remarkably very little information about its temporal genetic structure when it is growing in the intestine of mammals. Analysis of *E. coli* evolution within the human intestine started in the fifties, with longitudinal studies where clones of

*E. coli* isolated from feces of a human where collected periodically during several months and analyzed for variation at specific loci. These studies [8,9] suggested that there are strains of *E. coli* that can persist for months (resident strains) in the human gut and other strains that come and go (transient strains). Evidence for migration together with mutation and recombination shaping *E. coli* genetic structure, as well as for strain replacement, possibly due to the action of natural selection, has also been obtained.

However, important questions related to the characterization of natural within-host variation and to the strength of the evolutionary mechanisms shaping such variation have not yet been clearly answered. Some pertinent questions still remain: How many strains of *E. coli* are present within a host at any given time and how fast the genetic composition changes? How many dominant strains accompany a host during its lifetime, and what is their evolutionary and ecological nature? And more generally: at what pace does *E. coli* typically evolve in the mammalian gut, what are the major environmental forces shaping its evolution and under what key evolutionary mechanisms?

Future time series studies of the changes in genetic structure of both *E. coli* strains and the other species of the human microbiota should be very helpful in elucidating these issues. However, the relative role of the different ecological and evolutionary forces that shape *E coli* natural variation may be difficult to assess quantitatively in such complex environment. This is so because, as the previous studies indicate, many mechanisms may be at play simultaneously. In this respect animal models may turn out to be useful, as they allow specific mechanisms and hypothesis to be tested.

***Experimental evolution to dissect evolutionary change E. coli in the mammalian gut***

One way to start addressing one of the most basic questions: how fast do *E. coli* evolve in the mammalian gut?; is to perform experimental evolution (EE) *in vivo*. The dynamics of adaptation can be dissected with exquisite quantitative power by EE, a methodology where evolution in controlled environments is studied while it is occurring [10]. The experiments are designed such that theoretical predictions can be tested and important evolutionary parameters, such as the rate at which beneficial mutations occur and their effects on fitness, measured [11]. While EE to study *E. coli* adaptation in simple laboratory environments imposing specific selection pressures has led to a rich understanding of the adaptive process [12], much less is known about its adaptation in a more natural ecosystem. A great difference may be expected when one moves from a simple environment, where *E. coli* grows alone, to a complex one where host factors and other microbial species may influence its adaptation. In this respect there are two relevant ecological models to study the adaptation of *E. coli* to the gut: 1) the germ free mouse model, which mimics the initial process of *E. coli in vivo* evolution, as it is usually the first colonizer of the mammalian intestine of newborns [1], an initially sterile environment; 2) the streptomycin-treated mice, which mimics *E. coli* colonization when it competes with the major players of the mammalian microbiota, namely many Bacteriodetes and some

Firmicutes, [13, Xavier KB unpublished data], and also mimics conditions which often occur as a result of antibiotic treatment.

One of the key traits controlling genetic variation within a species is the mutation rate. The mutation rate of most bacteria is in the order of $10^{-3}$ per genome per generation, irrespectively of genome size [14]. Yet in many bacteria species, including *E. coli*, mutator strains, which exhibit an increased mutation rate due to mutations in DNA repair genes, can be found. Experiments in germ free mice colonized with either wild-type, mutator strains, or mixtures of both have revealed key insights to our understanding of the natural polymorphism for bacterial mutation rates [15]. *E. coli* mutator strains can emerge and increase in frequency during long-term colonization of germ-free mice. Such mutators invade not due to an intrinsic advantage (*i.e.* the mutator allele is not beneficial *per se*), but by their ability to hitchhike with the beneficial mutations they produce at higher per capita rates. However these benefits also entail a long-term cost. *In vivo* evolved mutator strains tend to accumulate many mutations, which are deleterious in *ex vivo* environments [15]. This cost selects against mutators and may keep the mutation rate low in natural populations [14]. The success of mutators observed in the gut of germ-free mice suggests that beneficial mutations are very common in this simplified environment. This conjecture was further supported by **the observation that** *E. coli* phenotypic diversity emerges rapidly, as evidenced by colonies with different morphologies and motilities, within a week of colonization of germ-free mice [16].

Experiments in streptomycin treated mice have also allowed further understanding of the physiological state of *E. coli* in the gut. Selection of mutants in the streptomycin-treated mouse intestines lead to the identification of beneficial mutations responsible for its increased colonization ability in this complex ecosystem [17,18]. These studies lead to the identification of important metabolic properties required for *E. coli* gut colonization in the presence of its competitors.

Given the previous evidence for rapid adaptation in the gut, a recent study sough to test if the pattern of *E. coli* gut evolution was supportive of the classical Fisher-Muller evolutionary mechanism – also known as clonal interference (CI) - which is driven by a large supply of beneficial mutations into evolving populations [19]. In such a scenario, the speed of adaptation **is expected to be** limited, a great number of weak beneficial mutations lost and mechanisms that allow for recombination to evolve. The study traced the occurrence of adaptive mutations in real time, by colonizing 15 streptomycin-treated mice with a co-culture of two strains of *E. coli*, each marked by a chromosomally encoded fluorescence and otherwise genetically identical. Evidence for very intense CI occurring in the gut was obtained first through following the changes in frequency of the fluorescent clones along time and next through direct competition of the evolved bacteria against the ancestral strain in newly colonized mice. The predictability of evolution was remarkable at the phenotypic level, with 15 out of 15 *E. coli* populations independently evolving inability to metabolize galactitol, a compound that *E. coli* may encounter in the gut and that was toxic to the initial colonizing strain. In contrast to such phenotypic sweeps, much variation could be detected at the

genetic level, caused by the emergence of strong mutations, at the *gat* operon, with similar fitness effects, in the different fluorescence backgrounds. Following the first burst of adaptive diversity, which happened in the first week post-colonization, further adaptive mutations occurred. These led to the increased frequency of haplotypes carrying more than one beneficial mutation. It also led to the elimination of beneficial gat alleles that were unlucky not to get linked to a secondary adaptive mutation – a phenomenon called soft sweeps. High degree of parallelism was also observed among the second adaptive mutations and the type of mutations identified reflect a metabolic optimization to the streptomycin treated gut environment. The study provided the first estimate of the genomic beneficial mutation rate ($> 7x10^{-7}$) and direct evidence for mutations with large fitness benefits (7%) in this ecosystem. *It revealed that the first steps of E. coli adaptation to the gut are not limited by mutation but limited by selection.*

Because the strength of the first and secondary mutations (Gordo, unpublished results) were similar, this study raises an important question to be addressed in the future: Is the rate of *E. coli* evolution in the gut constant or does it change with time?

The striking degree of parallelism observed for the first phenotypic sweep (gat phenotype) and the secondary adaptive mutations, highlights the power of this methodology. Next, the streptomycin-model of infection can be used in conditions that mimics disease associated with intestinal inflammation and loss of colonization resistance towards pathogens [20]. The same methodology can be applied to systematically analyze the role of components involved in gut homeostasis: the microbiota by using gnotobiology techniques (germ free mice colonized with specific members of the microbiota) or the host immune system by using mouse mutants affected in different players of the immune responses. The quantitative analysis of bacterial adaptive process under these different conditions will provide mechanistic understanding relevant for disease etiology and therapy.

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