

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

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7 KEYWORDS

8 • *Escherichia coli* • adaptation • gut microbiota • clonal interference

9 *Escherichia coli* is an extremely versatile species with a high adaptation
10 capacity to new and variable niches. It harbors an astonishing level of genetic
11 and phenotypic diversity and can even assume the form of a deadly
12 pathogen. But most members of the species live as commensals. Indeed *E.*
13 *coli* is commonly sampled from the feces of many mammals and birds, and it
14 is the predominant facultative anaerobic bacteria in the gastrointestinal tract.
15 In humans it colonizes the gut within hours after birth [1] and is a typical
16 stable inhabitant of our intestines, where it competes with other species of the
17 microbiota. In the seventies Milkman analyzed hundreds of natural *E. coli*
18 isolates and used multilocus enzyme electrophoresis to reveal an average
19 genetic diversity of 0.23 in 5 loci [2], a value that later revealed to be a lower
20 bound with the analysis of further loci [3]. Genetic variation in *E. coli*, like in
21 many other bacterial species is likely the result of the well known evolutionary
22 mechanisms: mutation, genetic drift, recombination, migration and natural
23 selection. While mutation, the primary mechanism of generation of new
24 alleles, genetic drift, the random sampling of alleles from one generation to
25 next, recombination, the exchange of genes between different strains, and
26 migration of clones between hosts are key processes which may account for
27 some features of the observed *E. coli* population genetic structure and
28 variation [4,5], natural selection is also thought to play a significant role. When
29 a new advantageous mutation emerges in a given gene and increases in
30 frequency, eventually fixing in the population (selective sweep), it leaves a
31 signature on the pattern of nucleotide variation at nearby sites. In particular if
32 selection is strongly favoring a beneficial mutation, the linked neutral alleles
33 will hitchhike with it and genetic variation will be wiped out following such
34 sweep [6]. Indeed the first suggestion of a global selective sweep in *E. coli*
35 came from the analysis of levels of polymorphism of its *gapA* gene which
36 exhibits a striking reduced variability in natural isolates of *E. coli*, amongst
37 other observed patterns departing from the expectations of neutral evolution
38 [7].

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

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

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

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

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

68 ***Experimental evolution to dissect evolutionary change E. coli in the*** 69 ***mammalian gut***

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

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

92 One of the key traits controlling genetic variation within a species is the
93 mutation rate. The mutation rate of most bacteria is in the order of 10^{-3} per
94 genome per generation, irrespectively of genome size [14]. Yet in many
95 bacteria species, including *E. coli*, mutator strains, which exhibit an increased
96 mutation rate due to mutations in DNA repair genes, can be found.
97 Experiments in germ free mice colonized with either wild-type, mutator strains,
98 or mixtures of both have revealed key insights to our understanding of the
99 natural polymorphism for bacterial mutation rates [15]. *E. coli* mutator strains
100 can emerge and increase in frequency during long-term colonization of germ-
101 free mice. Such mutators invade not due to an intrinsic advantage (*i.e.* the
102 mutator allele is not beneficial *per se*), but by their ability to hitchhike with the
103 beneficial mutations they produce at higher per capita rates. However these
104 benefits also entail a long-term cost. *In vivo* evolved mutator strains tend to
105 accumulate many mutations, which are deleterious in *ex vivo* environments
106 [15]. This cost selects against mutators and may keep the mutation rate low in
107 natural populations [14]. The success of mutators observed in the gut of
108 germ-free mice suggests that beneficial mutations are very common in this
109 simplified environment. This conjecture was further supported by **the**
110 **observation that** *E. coli* phenotypic diversity emerges rapidly, as evidenced
111 by colonies with different morphologies and motilities, within a week of
112 colonization of germ-free mice [16].

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

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

138 genetic level, caused by the emergence of strong mutations, at the *gat*
139 operon, with similar fitness effects, in the different fluorescence backgrounds.
140 Following the first burst of adaptive diversity, which happened in the first week
141 post-colonization, further adaptive mutations occurred. These led to the
142 increased frequency of haplotypes carrying more than one beneficial
143 mutation. It also led to the elimination of beneficial *gat* alleles that were
144 unlucky not to get linked to a secondary adaptive mutation – a phenomenon
145 called soft sweeps. High degree of parallelism was also observed among the
146 second adaptive mutations and the type of mutations identified reflect a
147 metabolic optimization to the streptomycin treated gut environment. The study
148 provided the first estimate of the genomic beneficial mutation rate ($> 7 \times 10^{-7}$)
149 and direct evidence for mutations with large fitness benefits (7%) in this
150 ecosystem. *It revealed that the first steps of E. coli adaptation to the gut are*
151 *not limited by mutation but limited by selection.*

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

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

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