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FEATURE ARTICLE

THE NATURAL HISTORY OF MODEL ORGANISMS

The unexhausted potential of *E. coli*

Abstract *E. coli*'s hardiness, versatility, broad palate and ease of handling have made it the most intensively studied and best understood organism on the planet. However, research on *E.coli* has primarily examined it as a model organism, one that is abstracted from any natural history. But *E. coli* is far more than just a microbial lab rat. Rather, it is a highly diverse organism with a complex, multi-faceted niche in the wild. Recent studies of 'wild' *E. coli* have, for example, revealed a great deal about its presence in the environment, its diversity and genomic evolution, as well as its role in the human microbiome and disease. These findings have shed light on aspects of its biology and ecology that pose far-reaching questions and illustrate how an appreciation of *E. coli*'s natural history can expand its value as a model organism.

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Introduction

In 1884, the German microbiologist and pediatrician Theodor Escherich began a study of infant gut microbes and their role in digestion and disease. During this study, he discovered a fast-growing bacterium that he called *Bacterium coli commune*, but which is now known as the biological rock star that is *Escherichia coli* (**Escherich, 1988**; **Shulman et al., 2007**; **Zimmer, 2008**). *E. coli*'s meteoric rise and exalted status in biology stem from how easy it is to find and work with. Hardy, non-pathogenic, and versatile strains that grow quickly on many different nutrients can be isolated from virtually any human. These traits made *E. coli* a mainstay in microbiology teaching lab collections. Consequently, when early 20th century microbiologists cast about for a model organism, *E. coli* was one of the most widely available choices.

Those who chose to work with *E. coli* included **Bordet and Ciuca (1921)**, **Werkman (1927)**, **Wollman (1925)**, **Wollman and Wollman (1937)** and **Bronfenbrenner and Korb (1925)**, **Bronfenbrenner (1932)**, who between them performed groundbreaking studies on bacterial physiology, viruses, and genetics (**Daegelen et al., 2009**). By the 1940s, its use in many foundational studies firmly established *E. coli* as *the* bacterial model organism of choice, making it the obvious organism to work with at the onset of the molecular biology revolution in the 1950s. As a result, it became the organism in which the most basic aspects of life, including the genetic code, transcription, translation, and replication, were first worked out (**Crick et al., 1961**; **Nirenberg et al., 1965**; see **Judson, 1996** for an excellent history of early molecular biology and *E. coli*'s role in it). The resulting knowledge and molecular methods for investigating and manipulating its biology have since led to *E. coli*'s prominence in academic and commercial genetic engineering, pharmaceutical production, and experimental microbial evolution (see **Box 1** for a glossary of specialist terms used in this article), not to mention the biotechnology industry, which contributed $500 billion to the global economy in 2011 (**Cohen et al., 1973**, **Schaechter and Neidhardt, 1987**; **Lenski, 2004**; **Bruschi et al., 2011**; **Kamionka, 2011**; **Huang et al., 2012**; **Kawecki et al., 2013**). It is not hyperbole to say that *E. coli* is now the most important model organism in biology (**Zimmer, 2008**; see **Box 2**).

For all of its importance, *E. coli* is quite nondescript. It is a fairly typical Gram-negative bacillus (see 'Glossary'), measuring only about 1 μm long by 0.35 μm wide, although this can

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Box 1. Glossary

Accessory genes—Genes that are not among the invariant core genome of a microbe, and are thus not present in all strains of a given species. Accessory genes are thought to improve an organism's fitness in a particular environmental or ecological context.

Biofilm—A group of microbes that grow together while adhering to each other and to a surface. Biofilms typically contain complex, diverse communities embedded in an extracellular, gelatinous matrix of polysaccharides, proteins, and DNA.

Experimental microbial evolution—A recently developed field of biology in which experiments with fast-growing and evolving populations of microorganisms are used to investigate evolutionary questions that cannot be addressed with slow-growing, larger organisms.

Flexible genome—The set of genes within a microbe's genome that are not ubiquitous in a species, but instead vary from strain to strain within that species. Typically, the flexible genome is larger than the core genome. Also called the dispensable, accessory, or adaptive genome.

Gram-negative—A diverse group of bacteria that have two membranes that regulate the entry of substances into and out of the cell, between which is a rigid cell wall that maintains the cell's shape and structural integrity. The name comes from the failure of these bacteria to retain crystal violet dye during the Gram-stain procedure.

Hemolytic anemia—Anemia caused by abnormal breakdown of red blood cells. In cases of *E. coli* O157:H7 infection, hemolytic anemia is caused by red blood cells being fragmented by blood clots that form in the capillaries.

Microbiome—The total microbial community that lives on and within the body of a large, multi-cellular organism like a human. The gut microbiome is typically by far the largest component of an organism's total microbiome.

Pan-genome—The complete set of all genes found among all strains of a microbial species.

Pathotype—A group of pathogenic strains of *E. coli* that cause disease in the same part of the body and via the same mechanism.

Restriction Enzyme—A DNA-degrading enzyme that recognizes and cleaves DNA at or near a particular sequence referred to as a 'restriction site'. Bacteria produce restriction enzymes to defend against viruses by degrading their DNA upon its insertion into the cell. Also called a 'restriction endonuclease'.

Shiga-like Toxin—A protein toxin produced by enterohemorrhagic *E. coli* that binds to particular receptors on the surfaces of epithelial cells in small blood vessels, mainly in the kidney, intestines, and lungs. Once in a cell, it inhibits protein synthesis and causes the cell to die (***Griffin and Tauxe, 1991***).

Thrombocytopenia—A lack of platelets in the blood, which reduces the ability of blood to clot. In *E. coli* O157:H7 infections, it is caused by large numbers of platelets being used up in small blood clots that form in the capillaries.

Virome—The sum total of all viruses that exist within or on an organism, including those within the microbiome, and those integrated into the organism's genome.

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vary considerably depending on the strain and its conditions. Even at high magnification it looks like nothing more than a tiny sausage (***Figure 1A***). It may have whip-like flagella that it uses to move about its environment, or hair-like pili that allow it to attach to surfaces or to other cells (***Figure 1B***). Physiologically, it is a facultative aerobe, meaning that it can grow happily with or without oxygen, but it cannot grow at extremes of temperature or pH nor can it degrade dangerous pollutants, photosynthesize, or do a variety of other things that interest microbiologists. Phylogenetically, it is a member of the Enterobacteriaceae, and is closely related to such pathogens as *Salmonella*, *Klebsiella*, *Serratia*, and the infamous *Yersinia pestis*, which causes plague (***Brenner and Farmer, 2007***).

The helpful lodger: *E. coli*'s relationship(s) with its hosts

In nature, *E. coli* is principally a constituent of the mammalian gut microbiome (see 'Glossary'), but it is also found, albeit less commonly, in the gut microbiomes of birds, reptiles and fish, as well as in soil, water, plants, and food (***Hartl and Dykhuizen, 1984***; ***Leimbach et al., 2013***). Its mammalian abode is why *E. coli* can metabolize lactose, the control of which was the subject of seminal studies of gene regulation (***Jacob et al., 1960***; ***Jacob and Monod, 1961***). *E. coli* is typically the most common aerobe in the lower intestine of mammals. However, the gut is primarily an anoxic environment, and the extremely large, highly diverse (500+ taxa) gut microbial community is dominated by obligate

Box 2. The contributions of *E.coli* to biology, medicine and industry

Research using *E. coli* has led to many advances in a variety of fields. The following is a sample of these fields, and the contributions this work has made. Citations are non-exhaustive and to key literature only.

Molecular Biology, Physiology, and Genetics: Elucidation of the genetic code (***Crick et al., 1961***), DNA replication (***Lehman et al., 1958***), transcription (***Stevens, 1960***), life cycle of lytic and lysogenic bacterial viruses (***Ellis and Delbrück, 1939***; ***Lwoff, 1953***), gene regulation (***Jacob et al., 1960***; ***Jacob and Monod, 1961***; ***Englesberg et al., 1965***), discovery of restriction enzymes (***Linn and Arber, 1968***; ***Meselson and Yuan, 1968***), characterization and study of persister variants (***Hu and Coates, 2005***; ***Hansen et al., 2008***; ***Lewis, 2010***; ***Amato et al., 2013***; ***Amato and Brynildsen, 2014***) and swarming motility behavior (***Harshey and Matsuyama, 1994***; ***Harshey, 2003***; ***Inoue et al., 2007***; Partridge and Harshe, 2013a), and elucidation of the structure and function of ATP synthase (***Capaldi et al., 2000***).

Pharmaceuticals: In vivo synthesis of recombinant therapeutic proteins, including insulin (to treat diabetes), interleukin-2 (metastatic melanoma), human interferon-β (multiple sclerosis), erythropoietin (anemia), Human growth hormone (pituitary disorders, short stature, muscle wasting), human blood clotting factors (hemophilia), pegloticase (gout), taxol (cancer) and certolizumab (Crohn's disease) (reviewed in ***Kamionka, 2011***; ***Huang et al., 2012***).

Evolution: Demonstration of the random nature of mutations (***Luria and Delbrück, 1943***; ***Lederberg and Lederberg, 1952***). Principal model organism in experimental evolution (reviewed in ***Kawecki et al., 2013***), used to examine many issues, including the relationship between genomic evolution and adaptation (***Barrick et al., 2009***), evolutionary repeatability and the role of historical contingency in evolution (***Travisano et al., 1995***; ***Cooper et al., 2003***; ***Blount et al., 2008***; ***Meyer et al., 2012***), the origin of novel traits (***Blount et al., 2012***), long-term fitness trajectories (***Wiser et al., 2013***), effect of sexual recombination on adaptation (***Cooper, 2007***), and predatory–prey interactions (***Chao and Levin, 1977***; ***Lenski, 1988***; ***Meyer et al., 2010***, ***2012***).

Genetic Engineering and Biotechnology: Development of genetic engineering techniques and technologies, including molecular cloning and recombinant DNA (***Cohen et al., 1973***), allele replacement (***Link et al., 1997***; ***Herring et al., 2003***). Used to produce biofuels (***Liu and Khosla, 2010***; ***Janßen and Steinbüchel, 2014***), and industrial chemicals such as phenol (***Kim et al., 2014***), ethanol (***Hildebrand et al., 2013***), mannitol (***Kaup et al., 2004***), and a variety of others (***Chen et al., 2013***).

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anaerobes, such as members of Bacteroides and Firmicutes, which alone comprise ~90% or more of the total gut microbial population (***Bäckhed et al., 2005***; ***Eckburg et al., 2005***; ***Claesson et al., 2009***; ***Tenaillon et al., 2010***). By contrast, *E. coli* typically constitutes only 0.1–5% of the community, which partly reflects the fact that its niche is to be found in the relatively thin layer of mucus that lines the gut. In the mucus layer, *E. coli* grows in a complex, multi-species biofilm (see 'Glossary') in which it competes for an array of nutrients—one of the origins of its broad diet (***Chang et al., 2004***; ***Beloin et al., 2008***). This rich food supply enables *E. coli* to maintain population densities of 10^6–10^9 cells per gram of fecal matter despite unavoidable and regular bulk losses (***Savageau, 1983***; ***Chang et al., 2004***). In the human gut, the *E. coli* population typically includes a set of long-term residential strains, and also short-term transients that vary with diet, health, and with exposure to antibiotics (***Sears et al., 1950***; ***Savageau, 1983***). The human gut *E.coli* population also varies due to its constant, largely unknown interactions with the broader microbiome, and with the host and the host's vast virome (see 'Glossary'), against which it defends itself with restriction enzymes, which have become a key tool in molecular biology (see 'Glossary') (***Kasarjian et al., 2003***; ***Roberts, 2005***; ***Minot et al., 2011***; ***He et al., 2013***; ***Shoaie et al., 2013***; ***Virgin, 2014***).

A host organism carefully regulates its microbiome in poorly understood ways that have an impact on its health (***Schluter and Foster, 2012***). For instance, the human gut secretes immunoglobulin A, which appears to facilitate the formation of *E. coli* biofilms on the intestinal mucosa, suggesting that their presence is welcome (***Bollinger et al., 2003***). While long considered to have a commensal relationship with its host, in which *E. coli* secures food and a nice warm home while contributing little in return, it is increasingly clear that the host-*E. coli* relationship is really a mutualism. Indeed, *E. coli* benefits its host in a number of ways. It produces vitamin K and vitamin B12, both of which are required by mammalian hosts (***Bentley and Meganathan, 1982***; ***Lawrence and Roth, 1996***).

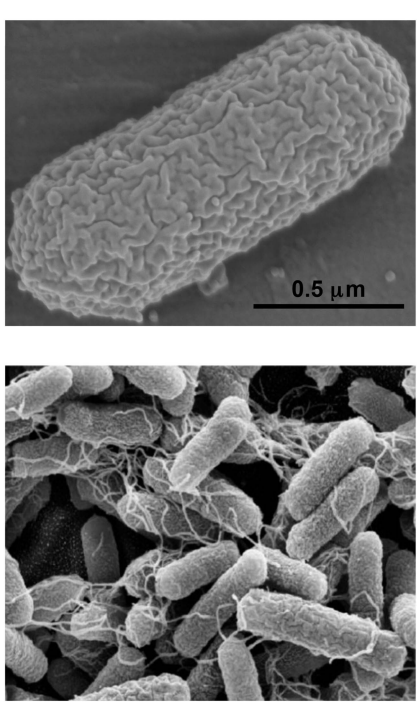

Figure 1. Scanning Electron Micrographs of *E. coli*. (**A**) *E. coli* B strain REL606, a laboratory strain with a typical sausage-shaped morphology. (Photo credit: Brian Wade). (**B**) *E. coli* O119:HND strain A111, an enteropathogenic strain that produces hair-like pili. (Photo credit: ***Nascimento et al., 2014***).
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E. coli also maintains a friendly environment for its anaerobic neighbors by consuming oxygen that enters the gut. Perhaps most importantly, *E. coli* competitively excludes pathogens from its niche in the gut, rather like how friendly barbarian tribes, settled by the Roman Empire on its frontiers, helped to keep out the more dangerous tribes (***Chang et al., 2004***).

E. coli's relationship with a host literally begins at birth. Newborns are typically inoculated with maternal *E. coli* through exposure to her fecal matter during birth and from subsequent handling (***Nowrouzian et al., 2003***; ***Leimbach et al., 2013***). Although perhaps disconcerting to ponder, this inoculation seems to be quite important. Indeed, *E. coli* becomes more abundant in the mother's microbiome during pregnancy, increasing the chances of her newborn's inoculation (***Koren et al., 2012***). The colonizing strains typically have secretion systems and pili that allow them to attach to and interact with the infant's gut epithelium (***Feeney et al., 1980***; ***de Muinck et al., 2013***). This newly established and rapidly growing *E. coli* population then changes the structure and function of the epithelial cells in ways that appear crucial for healthy microbiome development (***Tomas et al., 2015***). It is therefore concerning that early human infant colonization by *E. coli* has been declining in the US and in other Western nations as rates of caesarean delivery have increased and hospital hygiene has continued to improve (***Grönlund et al., 1999***; ***Nowrouzian et al., 2003***; ***Adlerberth, 2006***). Indeed, this decrease has been accompanied by broader microbiome changes, including increased infant gut colonization by *Staphylococcus aureus*, which is linked to an increased risk of developing a variety of disorders, including asthma, obesity, and diabetes (***Lindberg et al., 2000***, ***2004***; ***Neu and Rushing, 2011***; ***Sannasiddappa et al., 2011***; ***Rudi et al., 2012***; ***Azad et al., 2013***; ***Moeller et al., 2014***). The long-term consequences of disrupting humanity's long association with *E. coli* are under intensive investigation.

Life on the outside: *E. coli* in the external environment

An inevitable consequence of being a gut microbe is to be regularly excreted into the external world. The mucus lining of the gut is constantly sloughed off and excreted in fecal matter, so cells of a resident *E. coli* population are shed almost as soon as that population is established. *E. coli*'s long-term life cycle is hence biphasic, and despite being exquisitely adapted to the good life inside of a host, *E.coli* must also be adapted to successfully acclimate to a harsher life outside the host (***Savageau, 1983***). This is a remarkable feat. Whereas life on the inside is easy and stable, every aspect of the external environment, be it nutrition, temperature, oxygen, moisture, pH, and/or the surrounding microbial community, can fluctuate wildly (***Savageau, 1983***; ***Winfield and Groisman, 2003***; ***van Elsas et al., 2011***). It is likely that the hardiness, metabolic flexibility, and substrate breadth that have made *E. coli* such a valuable model organism evolved in part to permit it to survive this hostility and variability long enough to make it back to a host (***van Elsas et al., 2011***).

Another interesting trait that is almost certainly relevant to *E. coli*'s survival of its environmental phase is the production of persister variants. First observed in *Staphylococcus* during experiments with penicillin, persisters are rare, highly antibiotic-tolerant phenotypic variants that arise at random in bacterial populations (***Hobby et al., 1942***; ***Bigger, 1944***; ***Balaban et al., 2004***;

Box 3. Outstanding questions about the natural history of *E. coli*

- How do *E. coli* populations become established in soil and water communities outside of their host? What niches do they fill? How does adaptation to these new conditions affect their capacity to recolonize a host organism?
- How often do environmental strains find their way back to a host?
- What is the function of swarming motility in nature?
- What proportion of any given *E. coli* strain's complement of genes is adaptive to life in a host vs life in the external environment?
- How many different ecotypes of *E. coli* that occupy distinct niches are there?
- To what degree is *E. coli*'s genomic evolution in the wild driven by horizontal gene transfer vs mutation? How much horizontal gene transfer into *E. coli* comes from other organisms?
- How do the *E. coli* pan- and flexible genomes evolve, and what overlap is there with those of other organisms?
- On average, how many generations do wild *E. coli* strains undergo in a year?
- What is the average pace of evolution for wild *E. coli*? How does it vary between different strains occupying different hosts and environments?
- How tightly have *E. coli* and its hosts co-evolved?
- What impact does change in human lifestyles have on the relationship between humans and *E. coli*, and what are the health consequences?

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Lewis, 2010; **Zhang, 2014**). Persisters are not an adaptation specifically to antibiotics; their hallmark antibiotic tolerance is attributable to their metabolic inactivity, which is triggered by several redundant pathways, including those that govern stress response (**Shah et al., 2006**; **Lewis, 2010**; **Amato et al., 2013**; **Amato and Brynildsen, 2014**). Persistence therefore appears to be a general adaptation that permits small numbers of dormant cells to survive a variety of environmental fluctuations. *E. coli* has been, as with so many other phenomena, a model in which to study bacterial persistence, and it seems likely that this capacity to enter a dormant state plays a significant role in surviving the considerable fluctuations it encounters in its external environment.

The external world was long thought to be so harsh as to preclude *E. coli*'s growth outside of its host. While a tiny minority might eventually reach a new host, most cells were expected to eventually die. This is the basal assumption behind using the presence of *E. coli* as an indicator of fecal contamination. However, recent studies have shown that *E. coli* can, in fact, establish itself as a member of microbial soil, water, and plant-associated communities (**Lopez-Torres et al., 1987**; **Ishii and Sadowsky, 2008**; **Texier et al., 2008**; **Brennan et al., 2010**; **Berthe et al., 2013**; **Dublan et al., 2014**). Moreover, genomic and phylogenetic analyses of collections of *E. coli* strains have identified divergent lineages that appear to be adapted to a primarily non-host lifestyle (**Walk et al., 2009**). What adaptations are required for *E. coli* to make such radical ecological shifts, what niches it fills in its new communities, how stable its presence in those communities might be, and what impact its adaptation to these new niches might have on its capacity to return to a host remain outstanding questions that must be addressed (see **Box 3**).

It is possible that these environmental *E. coli* populations will help resolve the interesting problem of swarming motility. It has long been observed that groups of *E. coli* cells on water-restricted surfaces will congregate and engage in social, coordinated movement over the surface, a behavior also seen in other bacteria (**Harshey and Matsuyama, 1994**; **Harshey, 2003**; Partridge and Harshey, 2013a,b). However, swarming has principally been observed and studied on agar plates in the lab (Partridge and Harshey, 2013a). What function it might serve for *E. coli* in nature has been unclear. The gut generally lacks the sorts of surfaces on which swarming works. Given that ~216 *E. coli* genes are specifically involved in swarming motility, it is a costly trait that would not be maintained if it did not confer some selective benefit (**Inoue et al., 2007**). It is as yet unclear if swarming plays any role in the initial colonization of the gut, but it is associated with pathogenesis, likely due to its improved colonization of tissue surfaces that facilitate opportunistic infection (see the next section) (**Zhang et al., 2010**; Partridge and Harshey, 2013a). Given that surfaces, and interfaces between surfaces, are regularly encountered by environmental bacteria, swarming may well prove to be an adaptation that enables the exploration and colonization of viable habitats by *E. coli* upon their excretion into the external environment.

Pathogenic *E. coli*: A friendly microbe's dark side

E. coli's presence in the environment is a cause for concern because its relationship with humans is not entirely benign. Indeed, *E. coli* is a major cause of diarrheal diseases, peritonitis, colitis, bacteremia, infant mortality, and urinary tract infections that world-wide cost billions of dollars to treat and kill roughly 2 million humans each year (**Russo and Johnson, 2003**; **Kaper et al., 2004**). Some strains may even cause cancer (**Arthur et al., 2012**). Some opportunistic *E.coli* infections are caused by normally harmless or beneficial strains when introduced to sick hosts or to parts of a host's body outside of the gut (**Kaper et al., 2004**). However, there are also pathogenic strains that produce virulence factors and can cause illness in even the healthiest host. These strains are classified by where and how they cause disease into groups called pathotypes (see 'Glossary'), which include enteroaggregative, enterohemorrhagic, enteropathogenic, enterotoxigenic, uropathogenic, meningitis-associated, and septicemic-associated *E. coli* (**Kaper et al., 2004**; **Leimbach et al., 2013**) (see 'Glossary').

The most notorious of these is *E. coli* O157:H7, an enterohemorrhagic strain that produces a shiga-like toxin (**Griffin and Tauxe, 1991**; **Robinson et al., 2006**). This toxin (see 'Glossary') attacks small blood vessels, killing intestinal cells, and causing bloody diarrhea and severe abdominal pain, as well as hemolytic uremic syndrome (HUS), a potentially deadly condition that can involve widespread clots in capillaries and hemolytic anemia, thrombocytopenia, and renal failure (see 'Glossary'; **Griffin et al., 1988**; **Kaper et al., 2004**). Treatment can be difficult because antibiotics increase the risk of HUS. As a result, treatment is generally limited to the provision of fluids, adequate nutrition, medication for pain and fever, and blood transfusions when necessary (**Bitzan, 2009**; **Smith et al., 2012**).

O157:H7 is particularly dangerous because it can easily contaminate human food supplies. It resides asymptomatically in cattle and in other livestock, and can be transferred to humans via the fecal contamination of meat during its butchering and packaging (**Ferens and Hovde, 2011**). It can also contaminate vegetables via fertilizers and water, and through contact with live-stock-associated birds (**Callaway et al., 2009**, **2014**). Through these points of entry into the human food chain, O157 has caused numerous outbreaks of illness (**Frenzen et al., 2005**; **Rangel et al., 2005**). In the US alone, such outbreaks have annually affected ~63,000 individuals, killing 20, and costing around $405 million in healthcare and in lost productivity (**Mead et al., 1999**; **Scallan et al., 2011**). When *E. coli* gets bad press, O157:H7 is almost always the culprit, and rightfully so.

Diversity and plasticity of the *E. coli* genome

The many pathogenic strains of *E. coli* testify to the diversity of this single microbial 'species', the full extent of which was only revealed by the advent of whole-genome sequencing. For example, genome sequences have firmly placed all *Shigella* strains within the broader *E. coli* clade (**Pupo et al., 2000**; **Lukjancenko et al., 2010**; **Kaas et al., 2012**).

More importantly, sequencing has uncovered the remarkable plasticity and dynamism of the *E. coli* genome that contribute to its genetic and phenotypic diversity. In 2002, **Welch et al. (2002)** reported that three strains of *E. coli*, the popular lab strain K-12, O157:H7, and the uropathogenic strain CFT073, share only 39.2% of their genes. Subsequent sequencing of more strains has reduced this core genome to less than 20% of the more than 16,000 genes in the *E. coli* pangenome (see 'Glossary'; **Lukjancenko et al., 2010**; **Kaas et al., 2012**).

The remaining genes constitute the flexible genome (see 'Glossary'), a vast pool of 'plug and play' genetic variation that can be acquired via horizontal gene transfer (**Lukjancenko et al., 2010**; **Mira et al., 2010**; **Leimbach et al., 2013**). This variation includes prophages, transposable elements, and accessory genes (see 'Glossary'). These genes encode functions that can: improve fitness in particular niches; increase metabolic flexibility; and affect pathogenicity (**Schmidt and Hensel, 2004**; **Touchon et al., 2009**). Flexible genomic elements are often large and integrate into the genome at select insertion hotspots (**Touchon et al., 2009**). This capacity to mix and match accessory genetic elements means that new *E.coli* strains with novel combinations of traits can arise very quickly. Another consequence is that the size of the *E. coli* genome can vary greatly between strains. While standard lab strains have genomes of ~4.5 million base pairs and 4000 genes, pathogenic strains can have genomes of over 5.9 million base pairs and 5500 genes (**Blattner et al., 1997**; **Lukjancenko et al., 2010**; **de Muinck et al., 2013**).

This extensive genetic plasticity poses major questions for understanding how *E. coli* evolves

in the wild over the long-term. For instance, how does the rate of genomic evolution in a given *E. coli* lineage by mutation compare to that by horizontal gene transfer (**Guttman and Dykhuizen, 1994**; **Dolbrindt et al., 2010**; **Paul et al., 2013**)? Moreover, how much of the *E. coli* flexible genome ultimately derives from other organisms?

Conclusion

E. coli has been tremendously valuable as a decontextualized, abstracted model organism, but it could be of even greater value were we to gain a better understanding of its ecology and natural history. As I have discussed, it is a highly diverse and broadly distributed species that occupies an expansive, generalized niche in which it experiences a vast range of environmental and ecological conditions. This breadth presents two unique and synergistic opportunities. First, the tools and techniques developed for, and the knowledge derived from, the study of *E. coli*'s lab strains can be applied to studying its wild relatives in far greater detail than is possible for any other microbe. This capacity promises to yield new and profound insights into the biology of other microbes that experience similar conditions, as well as the discovery and identification of new microbiological phenomena. Second, any increase in our knowledge of *E. coli*'s natural history expands the range of biological phenomena for which its strains can be used as models to study. In other words, the study of *E. coli*'s natural history can reveal more than is possible with most organisms, and an increased understanding of its natural history in turn expands its potential as a model organism.

An example of this dynamic can be seen in studies of *E. coli* biofilms, which have revealed much about how harmless and pathogenic strains colonize the gut and persist in the environment (**Wang et al., 2006**; **Beloin et al., 2008**; **Nesse et al., 2014**; **Vogeleer et al., 2014**). These studies have, in turn, led to the development of *E. coli* as a model for studying the formation, genetics, physiology, and consequences of biofilms, generating important findings on this most common of microbial lifestyles (**Beloin et al., 2008**). Experimental evolutionary studies of *E. coli* biofilms have also led to findings of far-reaching consequence, such as how biofilms impact microbial evolution and how they facilitate the evolution of antibiotic resistance even when antibiotics are not present (**Ponciano et al., 2009**; **Tyerman et al., 2013**).

There are many unanswered questions about *E. coli*'s natural history (see **Box 3**). How does *E. coli* adapt to non-host environments? What role does it play in non-host communities? How does it adapt to life in the soil? Just how fluid is the *E. coli* genome? How do environmental and ecological conditions affect this fluidity? How does the pan-genome evolve? How much interaction is there between *E. coli*'s pan-genome and that of other microbes? How tightly adapted are hosts to their *E. coli* populations? How is *E. coli* adapting to changes in the human diet and lifestyle? Each unanswered question presents opportunities for novel research into unexplored corners of *E. coli*'s natural history, and the subsequent expansion of its potential as a model. Improved appreciation and interest in *E. coli*'s natural history can only uncover more questions, and increase its potential even more. If kept in mind by researchers, this dynamic will guarantee that the most important model organism of the 20th century will continue to be one of the most important model organisms of the 21st century and beyond.

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References

Adlerberth I, Lindberg E, Aberg N, Hesselmar SR, Strannegård IL, Wold A. 2006. Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? *Pediatric Research* **59**:96–101. doi: 10.1203/01.pdr.0000191137.12774.b2.

Amato SM, Brynildsen MP. 2014. Nutrient transitions are a source of persisters in *Escherichia coli* biofilms. *PLOS ONE* **9**:e93110. doi: 10.1371/journal.pone.0093110.

Amato SM, Orman MA, Brynildsen MP. 2013. Metabolic control of persister formation in *Escherichia coli*. *Molecular Cell* **50**:475–487. doi: 10.1016/j/molcel.2013.04.002.

Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Ting-Jia Fan, Bampbell BJ, Abujamel T, Dogan B, Rogers AB, et al. 2012. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* **338**:120–123. doi: 10.1126/science.1224820.

Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, Sears MR, Becker AB, Scott JA, Kozyrkyi AL, et al. 2013. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *Canadian Medical Association Journal* **185**:385–394. doi: 10.1503/cmaj.121189.

Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. 2005. Host-bacterial mutualism in the human intestine. *Science* **307**:1915–1920. doi: 10.1126/science.1104816.

Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. 2004. Bacterial persistence as a phenotypic switch. *Science* **305**:1622–1625. doi: 10.1126/science.1099390.

Barrick JE, Yu DS, Yoon SH, Oh TK, Schneider D, Lenski RE, Kim JF. 2009. Genome evolution and adaptation in a long-term experiment with *Escherichia coli*. *Nature* **461**:1243–1247. doi: 10.1038/nature08480.

Beloin C, Roux A, Ghigo JM. 2008. *Escherichia coli* biofilms. *Current Topics in Microbiology and Immunology* **322**:249–289. doi: 10.1007/978-3-540-75418-3_12.

Bentley R, Meganathan R. 1982. Biosynthesis of vitamin K (menaquinone) in bacteria. *Microbiological Reviews* **46**:241–280.

Berthe T, Ratajczak M, Clermont O, Denamur E, Petit F. 2013. Evidence for coexistence of distinct *Escherichia coli* populations in various aquatic environments and their survival in estuary water. *Applied and Environmental Microbiology* **79**:4684–4693. doi: 10.1128/AEM.00698-13.

Bigger JW. 1944. Treatment of *Staphylococcal* infections with penicillin. *Lancet* **244**:497–500. doi: 10.1016/S0140-6736(00)74210-3.

Bitzan M. 2009. Treatment options for HUS secondary *Escherichia coli* O157:H7. *Kidney International Supplement* **75**:S62–S66. doi: 10.1038/ki.2008.624.

Blattner FR, Plunkett G III, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, et al. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277**:1453–1462. doi: 10.1126/science.277.5331.1453.

Blount ZD, Barrick JE, Davidson CJ, Lenski RE. 2012. Genomic analysis of a key innovation in an experimental *Escherichia coli* population. *Nature* **489**:513–518. doi: 10.1038/nature11514.

Blount ZD, Borland CZ, Lenski RE. 2008. Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proceedings of the National Academy of Sciences of USA* **105**:7899–7906. doi: 10.1073/pnas.0803151105.

Bollinger RR, Everett ML, Palestrant D, Love SD, Lin SS, Parker W. 2003. Human secretory immunoglobulin A may contribute to biofilm formation in the gut. *Immunology* **109**:580–587. doi: 10.1046/j.1365-2567.2003.01700.x.

Bordet J, Ciuca M. 1921. Remarques sur l'historique des recherches, concernant la lyse microbienne transmissible. *Comptes Rendus Biologies* **84**:745–747.

Brennan FP, Abram F, Chinalia FA, Richards KG, O'Flaherty. 2010. Characterization of environmentally persistent *Escherichia coli* isolates leached from an Irish soil. *Applied and Environmental Microbiology* **76**:2175–2180. doi: 10.1128/AEM.01944-09.

Brenner DJ, Farmer JJ III. 2007. Family I. Enterobacteriaceae Rhn 1937, Nom. Fam. Cons. Opin. 15, Jud. Comm. 1958a, 73, Ewing, Farmer, and Brenner 1980, 674; Judicial Commission 1981, 104. In: Garrity G, Brenner DJ, Krieg NR, Staley JR. *Bergey's Manual of Systematic Bacteriology,* **Volume 2**. The Proteobacteria, Part B: The Gammaproteobacteria. New York: Springer. p. 587–850.

Bronfenbrenner JJ. 1932. The heat inactivation of bacteriophages. *Proceedings of the Society for Experimental Biology and Medicine* **29**:802–804. doi: 10.3181/00379727-29-6087.

Bronfenbrenner JJ, Korb C. 1925. Studies on the bacteriophage of d'Herelle: II. effect of alcohol on the bactiophage of d'Herelle. *Journal of Experimental Medicine* **42**:419–429. doi: 10.1084/jem.42.3.419.

Bruschi F, Dundar M, Gahan K, Gartland M, Szente M, Viola-Magni MP, Akbarova Y. 2011. Biotechnology worldwide and the 'European biotechnology Thematic Network' association (EBTNA). *Current Opinion in Biotechnology* **22**(Suppl 1):S7–S14. doi: 10.1016/j.copbio.2011.05.506.

Callaway TR, Carr MA, Edrington TS, Anderson RC, Nisbet DJ. 2009. Diet, *Escherichia coli* O157:H7, and cattle: a review after 10 years. *Current Issues in Molecular Biology* **11**:67–79.

Callaway TR, Edrington TS, Nisbet DJ. 2014. Isolation of *Escherichia coli* O157:H7 and *Salmonella* from migratory brown-headed cowbirds (*Molothrus ater*), common Grackles (*Quiscalus quiscula*), and cattle egrets (*Bubulcus ibis*). *Foodborne Pathogens and Disease* **11**:791–794. doi: 10.1089/fpd.2014.1800.

Capaldi RA, Schulenberg B, Murray J, Aggeler R. 2000. Cross-linking and electron microscopy studies of the structure and functioning of the *Escherichia coli* ATP synthase. *Journal of Experimental Biology* **203**:29–33.

Chang DE, Smalley DJ, Tucker DL, Leatham MP, Norris WE, Stevenson SJ, Anderson AB, Grissom JE, Laux DC, Cohen PS, et al. 2004. Carbon nutrition of *Escherichia coli* in the mouse intestine. *Proceedings of the National Academy of Sciences of USA* **101**:7427–7432. doi: 10.1073/pnas.0307888101.

Chao L, Levin BR. 1977. Complex community in a simple habitat: experimental study with bacteria and phage. *Ecology* **58**:369–378. doi: 10.2307/1935611.
Chen X, Zhou L, Tian K, Kumar A, Singh S, Prior BA, Wang Z. 2013. Metabolic engineering of *Escherichia coli*: a sustainable industrial platform for bio-based chemical production. *Biotechnology Advances* **31**: 1200–1223. doi: 10.1016/j.biotechadv.2013.02.009.
Claesson MJ, O'Sullivan O, Wang Q, Nikkilä J, Marchesi JR, Smidt H, de Vos WM, Ross RP, O'Toole PW. 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLOS ONE* **4**:e6669. doi: 10.1371/journal.pone.0006669.
Cohen S, Chang A, Boyer H, Helling R. 1973. Construction of biologically functional bacterial plasmids in vitro. *Proceedings of the National Academy of Sciences of USA* **70**:3240–3244. doi: 10.1073/pnas.70.11.3240.
Cooper TF. 2007. Recombination speeds adaptation by reducing competition between beneficial mutations in populations of *Escherichia coli*. *PLOS Biology* **5**: 1899–1905. doi: 10.1371/journal.pbio.0050225.
Cooper TF, Rozen DE, Lenski RE. 2003. Parallel changes in gene expression after 20,000 generations of evolution in *Escherichia coli*. *Proceedings of the National Academy of Sciences of USA* **100**:1072–1077. doi: 10.1073/pnas.0334340100.
Crick FH, Barnett L, Brenner S, Watts-Tobin RJ. 1961. General nature of the genetic code for proteins. *Nature* **192**:1227–1232. doi: 10.1038/1921227a0.
Daegelen P, Studier FW, Lenski RE, Cure S, Kim JF. 2009. Tracing ancestors and relatives of *Escherichia coli* B, and the derivation of B strains REL606 and BL21 (DE3). *Journal of Molecular Biology* **394**:634–643. doi: 10.1016/j.jmb.2009.09.022.
de Muinck EJ, Lagesen K, Afset JE, Didelot X, Rønningen KS, Rudi K, Stenseth NC, Trosvik P. 2013. Comparisons of infant *Escherichia coli* isolates link genomic profiles with adaptation to the ecological niche. *BMC Genomics* **14**:81. doi: 10.1186/1471-2164-14-81.
Dolbrindt U, Chowdary MG, Krumbholz G, Hacker J. 2010. Genome dynamics and its impact on evolution of *Escherichia coli*. *Medical Microbiology and Immunology* **199**:145–154. doi: 10.1007/s00430-010-0161-2.
Dublan MD, Ortiz-Marquez JCF, Lett L, Curatti L. 2014. Plant-adapted *Escherichia coli* show increased lettuce colonizing ability, resistance to oxidative stress and chemotactic response. *PLOS ONE* **9**:e110416. doi: 10.137/journal.pone.0110416.
Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargen M, Bill SR, Nelson KE, Relman DA. 2005. Diversity of the human intestinal microbial flora. *Science* **308**:1635–1638. doi: 10.1126/science.1110591.
Ellis EL, Delbrück M. 1939. The growth of bacteriophage. *Journal of General Physiology* **22**: 365–384. doi: 10.1085/jgp.22.3.365.
Englesberg E, Irr J, Power J, Lee N. 1965. Positive control of enzyme synthesis by gene C in the L-arabinose system. *Journal of Bacteriology* **90**:946–957.
Escherich T. 1988. The intestinal bacteria of the neonate and breast-fed infant (1885). *Review of Infectious Disease* **10**:1220–1225.
Feeney AR, Cooke EM, Shinebaum R. 1980. A comparative study of gram-negative aerobic bacilli in the faeces of babies born in hospital and at home. *Journal of Hygiene* **77**:129–139. doi: 10.1017/S0022172400026565.
Ferens WA, Hovde CJ. 2011. *Escherichia coli* O157:H7: Animal reservoir and sources of human infection. *Foodborne Pathogens and Disease* **8**:465–487. doi: 10.1089/fpd.2010.0673.
Frenzen PD, Drake A, Angulo FJ, The Emerging infections Program Foodnet working group. 2005. Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *Journal of Food Protection* **68**:2623–2630.
Griffin PM, Ostroff SM, Tauxe RV, Greene KD, Wells JG, Lewis JH, Blake PA. 1988. Illnesses associated with *Escherichia coli* O157:H7 infections. a broad clinical spectrum. *Annals of Internal Medicine* **109**:705–712. doi: 10.7326/0003-4819-109-9-705.
Griffin PM, Tauxe RV. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiologic Review* **13**: 60–98.
Grönlund MM, Lehtonen OP, Eerola E, Kero P. 1999. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *Journal of Pediatric Gastroenterology and Nutrition* **28**:19–25. doi: 10.1097/00005176-199901000-00007.
Guttman DS, Dykhuizen DE. 1994. Clonal divergence in *Escherichia coli* as a result of recombination, not mutation. *Science* **266**:1380–1383. doi: 10.1126/science.7973728.
Hansen S, Lewis K, Vulić M. 2008. Role of global regulators and nucleotide metabolism in antibiotic tolerance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **52**:2718–2726. doi: 10.1128/AAC.00144-08.
Harshey RM. 2003. Bacterial motility on a surface: many ways to a common goal. *Annual Reviews of Microbiology* **57**:249–273. doi: 10.1146/annurev.micro.57.030502.091014.
Harshey RM, Matsuyama T. 1994. Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. *Proceedings of the National Academy of Sciences of USA* **91**:8631–8635. doi: 10.1073/pnas.91.18.8631.
Hartl DL, Dykhuizen DE. 1984. The population genetics of *Escherichia coli*. *Annual Review of Genetics* **18**: 31–68. doi: 10.1146/annurev.ge.18.120184.000335.
He X, Mishchuk DO, Shah J, Weimer BC, Slupsky CM. 2013. Cross-talk between *E. coli* strains and a human colorectal adenocarcinoma-derived cell line. *Scientific Reports* **3**:3416. doi: 10.1038/srep03416.
Herring CD, Glasner JD, Blattner FR. 2003. Gene replacement without selection: regulated suppression of amber mutations in *Escherichia coli*. *Gene* **5**: 153–163. doi: 10.1016/S0378-1119(03)00585-7.
Hildebrand A, Schlacta T, Warmack R, Kasuga T, Fan Z. 2013. Engineering *Escherichia coli* for improved ethanol production from gluconate. *Journal of Biotechnology* **168**:101–106. doi: 10.1016/j.jbiotec.2013.07.033.
Hobby GL, Meyer K, Chaffee E. 1942. Observations on the mechanism of action of penicillin. *Experimental Biology and Medicine* **50**:281–285. doi: 10.3181/00379727-50-13773.
Hu Y, Coates AR. 2005. Transposon mutagenesis identifies genes which control antimicrobial drug tolerance in stationary-phase *Escherichia coli*. *FEMS*

Microbiology Letters **243**:117–124. doi: 10.1016/j.femsle.2004.11.049.
Huang C, Lin H, Yang X. 2012. Industrial production of recombinant therapeutics in *Escherichia coli* and its recent advancements. *Journal of Industrial Microbiology & Biotechnology* **39**:383–399. doi: 10.1007/s10295-011-1082-9.
Inoue T, Shingaki R, Hirose S, Waki K, Mori H, Fukui K. 2007. Genome-wide screening of genes required for swarming motility in *Escherichia coli*. *Journal of Bacteriology* **189**:950–957. doi: 10.1128/jb.01294-06.
Ishii S, Sadowsky MJ. 2008. *Escherichia coli* in the environment: implications for water quality and human health. *Microbes and Environments* **23**:101–108. doi: 10.1264/jsme2.23.101.
Jacob F, Monod J. 1961. Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology* **3**:318–356. doi: 10.1016/S0022-2836(61)80072-7.
Jacob F, Perrin D, Sanchez C, Monod J. 1960. L'opéron: groupe de génes à expression coordonnée par un opérateur (The operon: a group of genes whose expression is coordinated by an operator). *Comptes Rendus Hebdomadaires Des Séances De l'Académie Des Sciences* **250**:1727–1729. doi: 10.1016/j.crvi.2005.04.005.
Janßen HJ, Steinbüchel A. 2014. Fatty acid synthesis in *Escherichia coli* and its applications towards the production of fatty acid based fiofuels. *Biotechnology for Biofuels* **7**:7. doi: 10.1186/1754-6834-7-7.
Judson HF. 1996. *The Eighth Day of Creation.* Cold Spring Harbor: Cold Spring Harbor Press.
Kaas RS, Friis C, Ussery DW, Aarestrup FM. 2012. Estimating variation within the genes and inferring the phylogeny of 186 sequenced diverse *Escherichia coli* genomes. *BMC Genomics* **13**:577. doi: 10.1186/1471-2164-13-577.
Kamionka M. 2011. Engineering of therapeutic proteins production in *Escherichia coli*. *Current Pharmaceutical Biotechnology* **12**:268–274. doi: 10.2174/138920111794295693.
Kaper JB, Nataro JP, Mobley HLT. 2004. Pathogenic *Escherichia coli*. *Nature Reviews Microbiology* **2**:123–140. doi: 10.1038/nrmicro818.
Kasarjian JKA, Iida M, Ryu J. 2003. New restriction enzymes discovered from *Escherichia coli* clinical strains using a plasmid transformation method. *Nucleic Acids Research* **31**:e22. doi: 10.1093/nar/gng022.
Kaup B, Bringer-Meyer S, Sahm H. 2004. Metabolic engineering of *Escherichia coli*: construction of an efficient biocatalyst for D-mannitol formation in a whole-cell biotransformation. *Applied Microbiology and Biotechnology* **64**:333–339. doi: 10.1007/s00253-003-1470-9.
Kawecki TJ, Lenski RE, Ebert D, Hollis B, Olivieri I, Whitlock MC. 2013. Experimental evolution. *Trends in Ecology & Evolution* **27**:547–560. doi: 10.1016/j.tree.2012.06.001.
Kim B, Park H, Na D, Lee SY. 2014. Metabolic engineering of *Escherichia coli* for the production of phenol from glucose. *Biotechnology Journal* **9**:621–629. doi: 10.1002/biot.201300263.
Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Cling BH, Gonzalez A, Werner JJ, Angenent LT, Knight R, et al. 2012. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* **150**:470–480. doi: 10.1016/j.cell.2012.07.008.
Lawrence JG, Roth JR. 1996. Evolution of coenzyme B12 synthesis among enteric bacteria: evidence for loss and acquisition of a multigene complex. *Genetics* **143**:11–24.
Lehman IR, Bessman MJ, Simms ES, Kornberg A. 1958. Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *Escherichia coli*. *Journal of Biological Chemistry* **233**:163–170.
Leimbach A, Hacker J, Dobrindt U. 2013. *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. *Current Topics in Microbiology and Immunology* **358**:3–32. doi: 10.1007/82_2012_303.
Lewis K. 2010. Persister cells. *Annual Reviews of Microbiology* **64**:357–372. doi: 10.1146/annurev.micro.112408.134306.
Lindberg E, Adlerberth I, Hesselmar B, Saalman R, Strannegard IL, Aberg N, Wold AE. 2004. High rate of transfer of Staphylococcus aureus from parental skin to infant gut flora. *Journal of Clinical Microbiology* **42**:530–534. doi: 10.1128/JCM.42.2.530-534.2004.
Lindberg E, Nowrouzian F, Adlerberth I, Wold AE. 2000. Long-time persistence of superantigen-producing Staphylococcus aureus strains in the intestinal microflora of healthy infants. *Pediatric Research* **48**:741–747. doi: 10.1203/00006450-200012000-00007.
Link AJ, Phillips D, Church GM. 1997. Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: applications to open reading frame characterization. *Journal of Bacteriology* **179**:6228–9237.
Linn S, Arber W. 1968. Host specificity of DNA produced by *Escherichia coli*. X. In vitro restriction of phage fd replicative form. *Proceedings of the National Academy of Sciences of USA* **59**:1300–1306. doi: 10.1073/pnas.59.4.1300.
Liu T, Khosla C. 2010. Genetic engineering of *Escherichia coli* for biofuel production. *Annual Review of Genetics* **44**:53–69. doi: 10.1146/annurev-genet-102209-163440.
Lopez-Torres AJ, Hazen TC, Toranzos GA. 1987. Distribution and in situ survival and activity of Klebsiella pneumonia and *Escherichia coli* in a tropical rain forest watershed. *Current Microbiology* **15**:213–218. doi: 10.1007/BF01577533.
Lukjancenko O, Wassenaar TM, Ussery DW. 2010. Comparison of 61 sequenced *Escherichia coli* genomes. *Microbial Ecology* **60**:708–720. doi: 10.1007/s00248-010-9717-3.
Lederberg J, Lederberg E. 1952. Replica plating and indirect selection of bacterial mutants. *Journal of Bacteriology* **63**:399–406.
Lenski RE. 1988. Experimental studies of pleiotropy and epistasis in *Escherichia coli*. I. variation in competitive fitness among mutants resistant to virus T4. *Evolution* **42**:425–432. doi: 10.2307/2409028.
Lenski RE. 2004. Phenotypic and genomic evolution during a 20,000 generation experiment with the bacterium *Escherichia coli*. *Plant Breeding Reviews* **24**:225–265. doi: 10.1002/9780470650288.ch8.
Luria SE, Delbrück M. 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* **28**:491–511.
Lwoff A. 1953. Lysogeny. *Bacteriology Reviews* **17**:269–337.

Mead PS, Slutsker L, Dietz V, McCraig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* **5**:607–625. doi: 10.3201/eid0505.990502.
Meselson M, Yuan R. 1968. DNA restriction enzyme from *E. coli*. *Nature* **217**:1110–1114. doi: 10.1038/2171110a0.
Meyer JR, Agrawal AA, Quick RT, Dobias DT, Schneider D, Lenski RE. 2010. Parallel changes in host resistance to viral infection during 45,000 generations of relaxed selection. *Evolution* **60**:3024–3034. doi: 10.1111/j.1558-5646.2010.01049.x.
Meyer JR, Dobias DT, Weitz JS, Barrick JE, Quick RT, Lenski RE. 2012. Repeatability and contingency in the evolution of a key innovation in phage lambda. *Science* **335**:428–432. doi: 10.1126/science.1214449.
Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, Lewis JD, Bushman FD. 2011. The human gut virome: inter-individual variation and dynamic response to diet. *Genome Research* **21**:1616–1625. doi: 10.1101/gr.122705.111.
Mira A, Martin-Cuadrado AB, D'Auria G, Rodríguez-Valera F. 2010. The bacterial pan-genome: a new paradigm in microbiology. *International Microbiology* **12**:45–57. doi: 10.2436/20.1501.01.110.
Moeller AH, Li Y, Ngole EM, Ahuka-Mundeke S, Lonsdorf EV, Pusey AE, Peeters M, Hahn BH, Ochman H. 2014. Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences of USA* **111**:16431–16435. doi: 10.1073/pnas.1419136111.
Nascimento HH, Silva LE, Souza RT, Silva NP, Scaletsky IC. 2014. Phenotypic and genotypic characteristics associated with biofilm formation in clinical isolates of atypical enteropathogenic *Escherichia coli* (aEPEC) strains. *BMC Microbiology* **14**:184. doi: 10.1186/1471-2180-14-184.
Nesse LL, Sekse C, Berg K, Johannesen KC, Solheim H, Vestby LK, Urdahl AM. 2014. Potentially pathogenic *Escherichia coli* can form a biofilm under conditions relevant to the food production chain. *Applied and Environmental Microbiology* **80**:2042–2049. doi: 10.1128/AEM.03331-13.
Nowrouzian F, Hesselmar B, Saalman R, Strannegard I, Aberg N, Wold AE, Adlerberth I. 2003. *Escherichia coli* in infants' intestinal microflora: colonization rate, strain turnover, and virulence gene carriage. *Pediatric Research* **54**:8–14. doi: 10.1203/01.PDR.0000069843.20655.EE.
Neu J, Rushing J. 2011. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. *Clinics in Perinatology* **38**:321–331. doi: 10.1016/j.clp.2011.03.008.
Nirenberg M, Leder P, Bernfield M, Brimacombe R, Trupin J, Rottman F, O'Neal C. 1965. RNA codewords and protein synthesis, VII. On the general nature of the RNA code. *Biochemistry* **53**:1161–1168. doi: 10.1073/pnas.53.5.1161.
Partridge JD, Harshey RM. 2013a. More than motility: *Salmonella flagella* contribute to overriding friction and facilitating colony hydration during swarming. *Journal of Bacteriology* **195**:919–929. doi: 10.1128/jb.02064-12.
Partridge JD, Harshey RM. 2013b. Swarming: flexible roaming plans. *Journal of Bacteriology* **195**:909–918. doi: 10.1128/jb.02063-12.
Paul S, Linardopoulou EV, Billig M, Tchesnokova V, Price LB, Johnson JR, Chattopadhyay S, Sokurenko EV. 2013. Role of homologous recombination of extraintestinal *Escherichia coli*. *Journal of Bacteriology* **195**:231–242. doi: 10.1128/JB.01524-12.
Ponciano JM, La HJ, Joyce P, Forney LJ. 2009. Evolution of diversity in spatially structured *Escherichia coli* populations. *Applied and Environmental Microbiology* **75**:6047–6054. doi: 10.1128/AEM.00063-09.
Pupo GM, Lan R, Reeves PR. 2000. Multiple independent origins of Shigella clones of *Escherichia coli* and convergent evolution of many of their characteristics. *Proceedings of the National Academy of Sciences of USA* **97**:10567–10572. doi: 10.1073/pnas.180094797.
Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. 2005. Epidemiology of *Escherichia coli* O157:H7, 1982–2002. *Emerging Infectious Diseases* **11**:603–609. doi: 10.3201/eid1104.040739.
Roberts RJ. 2005. How restriction enzymes became the workhorses of molecular biology. *Proceedings of the National Academy of Sciences of USA* **102**:5902–5908. doi: 10.1073/pnas.0500923102.
Robinson CM, Sinclair JF, Smith MJ, O'Brien AD. 2006. Shiga toxin of enterohemorrhagic *Escherichia coli* type O157:H7 promotes intestinal colonization. *Proceedings of the National Academy of Sciences of USA* **103**:9667–9672. doi: 10.1073/pnas.0602359103.
Rudi K, Storrø O, Øien T, Johnsen R. 2012. Modelling bacterial transmission in human allergen-specific IgE sensitization. *Letters in Applied Microbiology* **54**:447–454. doi: 10.1111/j.1472-765X.2012.03229.x.
Russo TA, Johnson JR. 2003. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly endemic problem. *Microbes and Infection* **5**:449–456. doi: 10.1016/S1286-4579(03)00049-2.
Sannasiddappa TH, Costabile A, Gibson GR, Clarke SR. 2011. The influence of *Staphylococcus aureus* on gut microbial ecology in an in vitro continuous culture human colonic model system. *PLOS ONE* **6**:e23227. doi: 10.1371/journal.pone.0023227.
Savageau MA. 1983. *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. *The American Naturalist* **122**:732–744. doi: 10.1086/284168.
Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases* **17**:7–15. doi: 10.3201/eid1701.091101p1.
Schaechter M, Neidhardt FC. 1987. Indroduction. In: Neidhardt FC, editor. *Escherichia coli and Salmonella typhimurium*. Washington: American Society for Microbiology Press. p. 1–2.
Schluter J, Foster KR. 2012. The evolution of mutualism in gut microbiota via host epithelial selection. *PLOS Biology* **10**:e1001424. doi: 10.137/journal.pbio.1001424.
Schmidt H, Hensel M. 2004. Pathogenicity islands in bacterial pathogens. *Clinical Microbiology Reviews* **17**:14–56. doi: 10.1128/CMR.17.1.14-56.2004.
Sears HI, Brownlee I, Uchiyama JK. 1950. Persistence of individual strains of *E. coli* in the intestinal tract of man. *Journal of Bacteriology* **59**:293–301.

Shah D, Zhang Z, Khodursky AB, Kaldalu N, Kurg K, Lewis K. 2006. Persisters: a distinct physiological state of *E. coli*. *BMC Microbiology* **6**:53. doi: 10.1186/1471-2180-6-53.
Shoaie S, Kalrsson F, Mardinoglu A, Nookaew I, Bordel S, Nielsen J. 2013. Understanding the interactions between bacteria and the human gut through metabolic modeling. *Scientific Reports* **3**:2532. doi: 10.1038/srep02532.
Shulman ST, Friedmann HC, Sims RH. 2007. Theodor Escherich: the first pediatric infectious diseases physician? *Clinical Infectious Diseases* **45**:1025–1029. doi: 10.1086/521946.
Smith KE, Wilker PR, Reiter PL, Hedican EB, Bender JB, Hedberg CW. 2012. Antibiotic treatment of *Escherichia coli* O157 infection and the risk of hemolytic uremic syndrome, Minnesota. *Pediatric Infectious Disease Journal* **31**:37–41. doi: 10.1097/INF.0b013e31823096a8.
Stevens A. 1960. Incorporation of the adenine ribonucleotide into RNA by cell fractions from *E. coli*. *Biochemical and Biophysical Research Communications* **3**:92–96. doi: 10.1016/0006-291X(60)90110-8.
Tenaillon O, Skurnik D, Picard B, Denamur E. 2010. The population genetics of commensal *Escherichia coli*. *Natures Reviews Microbiology* **8**:207–217. doi: 10.1038/nrmicro2298.
Texier S, Prigent-Combaret C, Gourdon MH, Poirier MA, Faivre P, Dorioz JM, Poulenard J, Jocteur-Monrozier L, Moenne-Loccoz Y, Trevisan D. 2008. Persistence of culturable *Escherichia coli* fecal contaminants in dairy Alpine grassland soils. *Journal of Environmental Quality* **37**:2299–2310. doi: 10.2134/jeq2008.0028.
Tomas J, Reygner J, Mayeur C, Ducroc R, Bouet S, Bridonneau C, Cavin J, Thomas M, Langella P, Cherbuy C. 2015. Early colonizing *Escherichia coli* elicits remodeling of rat colonic epithelium shifty toward a new homeostatic state. *The ISME Journal* **9**:46–58. doi: 10.1038/ismej.2014.111.
Touchon M, Hoede C, Tenaillon O, Barbe V, Baeriswyl S, Bidet P, Bingen E, Bonascorsi S, Bouchier C, Bouvet O, et al. 2009. Organised genome dynamics in the *Escherichia coli* species results in high diverse adaptive paths. *PLOS Genetics* **5**:e1000344. doi: 10.1371/journal.pgen.1000344.
Travisano M, Mongold JA, Bennet AF, Lenski RE. 1995. Experimental tests of the roles of adaptation, chance and history in evolution. *Science* **267**:87–90. doi: 10.1126/science.7809610.
Tyerman JG, Ponciano JM, Joyce P, Forney LJ, Harmon LJ. 2013. The evolution of antibiotic susceptibility and resistance during the formation of *Escherichia coli* biofilms in the absence of antibiotics. *BMC Evolutionary Biology* **13**:22. doi: 10.1186/1471-2148-13-22.
van Elsas JD, Semenov AV, Costa R, Trevors JT. 2011. Survival of *Escherichia coli* in the environment: fundamental and public health aspects. *The ISME Journal* **5**:173–183. doi: 10.1038/ismej.2010.80.
Virgin HW. 2014. The virome in mammalian physiology and disease. *Cell* **157**:142–150. doi: 10.1016/j.cell.2014.02.032.
Vogeleer P, Tremblay YD, Mafu AA, Jacques M, Harel J. 2014. Life on the outside: role of biofilms in environmental persistence of Shiga-toxin producing *Escherichia coli*. *Frontiers in Microbiology* **5**:317. doi: 10.3389/fmicb.2014.00317.
Walk ST, Alm EW, Gordon DM, Ram JL, Toranzos GA, Tiedje JM, Whittam TS. 2009. Cryptic lineages of the genus *Escherichia*. *Applied and Environmental Microbiology* **75**:6534–6544. doi: 10.1128/AEM.01262-09.
Wang X, Rochon M, Lamprokostopoulou H, Lünsdorf H, Nimitz M, Römling U. 2006. Impact of biofilm matrix components of commensal *Escherichia coli* with the gastrointestinal cell line HT-29. *Cellular and Molecular Life Sciences* **63**:2352–2363. doi: 10.1007/s00018-006-6222-4t.
Welch RA, Burland V, Plunkett G III, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, et al. 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proceedings of the National Academy of Sciences of USA* **99**:17020–17024. doi: 10.1073/pnas.252529799.
Werkman CH. 1927. Vitamin effects in the physiology of microorganisms. *Journal of Bacteriology* **14**:335–347.
Winfield MD, Groisman EA. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology* **69**:3687–3694. doi: 10.1128/AEM.69.7.3687-3694.2003.
Wiser MJ, Ribeck N, Lenski RE. 2013. Long-term dynamics of adaptation in asexual populations. *Science* **342**:1364–1367. doi: 10.1126/science.1243357.
Wollman E. 1925. Recherches sur la bacteriophagie (phenomene de twort-d'Herelle). *Annales de l'Institut Pasteur* **56**:137–164.
Wollman E, Wollman E. 1937. The phases of bacteriophages. *Comptes Rendus Hebdomadaires Des Séances Et Mémoires De La Société De Biologie (Proceedings of the Academy of Sciences and Minutes of the Society of Biology)* **124**:931–934.
Zhang R, Turner L, Berg HC. 2010. The upper surface of an *Escherichia coli* swarm is stationary. *Proceedings of the National Academy of Sciences of USA* **107**:288–290. doi: 10.1073/pnas.0912804107.
Zhang Y. 2014. Persisters, persistent infections and the Yin-Yang model. *Emerging Microbes and Infections* **3**:e3. doi: 10.1038/emi.2014.3.
Zimmer C. 2008. *Microcosm*. New York: Pantheon Books.