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The evolution of E. coli is NOT driven by genetic variance but by thermodynamics.

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Abstract

> On the basis of empirical evidence, a) the evolution of *E. coli* is not driven by genetic variance, b) the laws that govern the evolution of *E. coli* are generic and apply to all natural systems, including galaxies, and c) genes are not Mendel's unit of inheritance.

Frontispiece

This paper is an update of "The Gene: An Appraisal" (*The Gene*) by K Baverstock, published in Progress in Biophysics and Molecular Biology (PBMB) in 2021. The paper challenges the functional role of the gene and, thereby, the Modern Synthesis, arguing that thermodynamics is the driver of evolution. (The Pre-proof of *The Gene*, as accepted for publication, is provided here since Elsevier's proofing software misnumbered some footnotes.)

On the publication of *The Gene,* the Editor-in-Chief of PBMB invited 45 researchers whose work was cited in the paper to submit commentaries; seven did so. None disputed the argument against the Modern Synthesis.

Thus, we continue to challenge the Modern Synthesis and invite the widest possible comment and criticism on the following work in progress.

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Introduction

Richard Lenski started the long-term evolution experiment (LTEE) in 1988 with a founder population of genetically pure *E. coli*. After over 30 years and 75,000 generations, it is still running (Lenski 2023). The LTEE comprises 12 independent replicates, i.e., identically treated cultures. Every 24 hours, a sample of each of the 12 cultures is transferred to a new flask containing fresh nutrients, which are "limited" insofar as they will all be consumed within the next 24 hours. Periodically, the fitness, i.e., the growth rate relative to that of the founder bacteria, and the size of the bacteria are measured. Less frequently, genome sequencing records the accrual of mutations.

The study provides a comprehensive body of evidence from which we conclude that the evolution of *E. coli* is NOT driven by genetic variance but by thermodynamics.

The Evidence

In 1994, after 5,000 generations of the LTEE, it was clear that each replicate of the 12 cultures evolved with a unique trajectory of increase in cell size (Lenski and Travisano 1994) (See Figure 2).

In 2009, the fitness profile and accrual of mutations were analysed for one of the 12 cultures. Unexpectedly, the authors failed to correlate the fitness profile to the accrued mutations using conventional genetics (Barrick, Yu et al. 2009). However, from Figure 2, it is clear that the rate of fitness increase was at a maximum when the accrual of mutations was at a minimum.

In 2013, when the experiment had been running for more than 50,000 generations, it was clear that the trajectory of the fitness profile fitted a power law rather than a hyperbolic law which might be expected if there were some physical limit to the fitness that could be achieved, leading to asymptotic behaviour. Strikingly, the 12 independent cultures exhibited identical power law trajectories (Wiser, Ribeck et al. 2013).

In 2020, it was confirmed that each replicate of the 12 cultures accrued its own unique spectrum of mutations, i.e., genetic variation (Maddamsetti and Grant 2020).

Conclusion 1

The improbability of all 12 LTEE cultures having identical fitness trajectories despite accruing diverse spectra of mutations means that Fisher's Genetical Theory of Natural Selection[1] is violated; the *E. coli* evolve independently of the mutations they are accruing. The evolution of 'fitness' differs from that of 'cell size', which is likely attributable to gene duplication (Bratlie, Johansen et al. 2010), i.e., a mutational event. In other words, fitness or adaptation in evolution is manifestly NOT driven by genetic variation.

Conclusion 2

The power law characteristic of fitness is the universal signature of the principle of least action (Makela and Annila 2010). Synonymous with the Second Law of Thermodynamics (hereinafter the 2nd Law), it governs the evolution of natural

systems. Since free energy is invariably consumed in the least time, the power law characteristic is found across scales and scopes, including genomes, proteomes, cultures, and ecosystems, as well as sandpiles, earthquakes, stars, and galaxies.

Conclusion 3

Although mutations are clearly inherited in the *E. coli* LTEE, they do not functionally influence the evolution of *E. coli* in terms of fitness, i.e., adaptation. Thus, genes cannot be Mendel's units of inheritance. Instead, the phenotypes of the gametes that fuse into the zygote must be accountable (*The Gene*, p 22).

Discussion

To the best of our knowledge, the *E. coli* LTEE is a unique experiment. Although it was not designed to test evolutionary hypotheses, it was well designed with 12 independent replicate cultures running in parallel in an identical environment and on identical support. We believe it is, therefore, legitimate to point out that this experiment falsifies Fisher's Genetical Theory of Natural Selection (Fisher 1930).

As noted above (The Evidence), the Modern Synthesis's failure was clear in 2009 from the results of one of the cultures (Barrick, Yu et al. 2009). Figure 2 in the 2009 paper shows the fitness trajectory and the accumulation of mutations in relation to the number of cell doublings. The fitness is increasing at its maximum rate while the accrued mutations (genetic variance) are very low, next to zero. Instead of acknowledging this glaring contradiction to their expectations, the authors fruitlessly seek an explanation in terms of the contemporary genetic theory, the Modern Synthesis[2] or Neo-Darwinian Theory.

Furthermore, geneticists widely assume that the majority of mutations are deleterious, not beneficial, by a large margin, even 1,000,000 to 1 (Elena and Lenski 1998). Accordingly, one would expect that, initially, fitness would *decrease* with each generation. Indeed, as discussed in *The Gene* (p 17 et seq.), in simulations of Fisher's law (Basener and Sandford 2018), fitness declines, and, with a very large excess of deleterious mutations, the population goes extinct.

By 2013, it was unambiguous that fitness evolved independently of the mutational yield (Wiser, Ribeck et al. 2013). The 12 independent cultures have identical fitness trajectories despite diverse spectra of mutations. That the trajectory is a power law rather than a hyperbolic law surprises the authors, who say: "The power law describes the fitness trajectories well, but it is not explanatory.". However, that is not the case. The generic trajectory for the evolution of *any* natural system, animate or inanimate, trends a power law (Makela and Annila 2010). It is an inevitable consequence of evolution governed by the principle of least action, derived by Maupertuis in 1740. This principle is synonymous with the 2nd Law of Thermodynamics. It says a system will minimize free energy, by whatever efficient means and mechanisms it has, to attain thermodynamic balance with its environment in the least time.

To be precise, the power law is a central approximation of the sigmoidal curve: fitness increases with every doubling of

the bacterial population, but that increase steadily diminishes. Looking for an explanation of this kind of trajectory, Wiser et al. propose two genetic features, 'diminishing returns epistasis' and 'clonal interference', to model theoretical 'mean-fitness trajectories to fit the experimental data' (Wiser, Ribeck et al. 2013). However, known as instrumentalism, such modelling after the fact only mimics data but does not explain the data[3].

Epistasis has frequently been invoked to bring experimental results into line with theory ever since its introduction in 1909 by William Bateson. Despite this extensive use, no clear mechanism has been identified to account for the postulated interference between two mutations due to their interactions with each other or their interactions with the genetic background. Indeed, Jason Moore wrote in *Human Hereditary* in 2003, 'What is a proper method for detecting epistasis? The answer to this question is currently unknown....' (Moore 2003).

As far as we can discover, there is no definitive empirical evidence relating to how epistasis is supposed to occur. Early studies (Elena and Lenski 1997) with *E. coli* were at odds with epistasis producing increases in fitness by showing a decline in fitness with increasing numbers of mutations. Specifically, we ask why epistasis would produce identical profiles for fitness but not for cell size.

We see no way to rescue the Modern Synthesis; the results of the LTEE must be accepted at their face value. Thus, Conclusion 1, genetic variation does NOT drive evolution, and Conclusion 2, evolution is a thermodynamic process, are solidly supported by the identical fitness trajectories (Wiser, Ribeck et al. 2013) arising from diverse mutational spectra (Maddamsetti and Grant 2020) in 12 independent replicate cultures. Run over more than 50,000 generations, the power law trajectory of fitness emerges unambiguously as the hallmark of evolution.

Conclusion 3, genes cannot be Mendel's units of inheritance, follows from Conclusions 1 and 2. At the time Mendel was experimenting, he knew nothing of what are now termed the genotype and genes. These concepts were introduced by Wilhelm Johannsen (Johannsen 1911, reprint Johannsen 2014) in the early 1900s, only after Mendel's 1865 and 1866 papers proposing the laws of inheritance were introduced to the scientific community by Hugo De Vries in 1900.

Let us recall Mendel's experiments on flower colour in pea plants to recognize the mechanism behind Mendel's laws. The wild-type colour of the plant's flowers is purple, and that of the mutant is white. The mutant genotype lacks an effective gene (a transcription factor) to switch on the synthesis of the purple dye (Hellens, Moreau et al. 2010). Thus, in this case, the mutation is the *lack* of a functional protein. It is a very rare category of mutation occurring in an organism but is maintained in a population by inheritance. In human populations, such mutations, a minor component of genetic variation, are responsible for rare inherited diseases, not common ones. Most mutations are single nucleotide polymorphisms (alternate alleles). This major component of genetic variation is *not* driving evolution because although the gene sequence change is inherited, it does not influence the cellular phenotype. If it did, it would be driving evolution, but the LTEE shows that it does not.

Thus, it is clear that Mendel's laws of inheritance are based on a special rare category of mutations. Accordingly, his laws should not be assumed to account for the so-called genetic component of common diseases, believed to be more than 50% based on the basis of family and twin studies (Plomin 2018)[4]. However celebrated it might be, the Mendelian

assumption of genes as the units of inheritance is wrong.

Despite its breakdown, the Mendelian assumption underpins a huge amount of Genome-Wide Association Studies (GWAS) attempting to attribute heritable single nucleotide polymorphisms or alternative alleles to common diseases. Since 2002, 41,000 papers have been published with “genome-wide association”/ “GWA” in the title or abstract, some 25,000 of them in the last decade and 11,000 since 2020. Notably, 847 of those latter papers have been published in journals with ‘Nature’ in the title. However, as noted by (Panath and Vermond 2018), GWA studies have not contributed to measurable public health advances.

The failure of experimental genetics to fully comply with Mendel’s laws has not prompted the genetics community to question the foundations of their discipline but rather, has led them to devise ‘features’ such as epistasis, under dominance, over dominance, etc., to bring the theory in line with the experimental results. The superfluous GWA studies, overseen by leading journals, parade an unfortunate failure in due diligence and preservation of the integrity of science.

Overall Conclusion

The LTEE has overturned Johannsen’s genotype conception and Fisher’s Genetical Theory of Natural Selection, underpinning Modern and Extended Syntheses, the mainstream evolution and population genetics theories. The former is the sole justification for Genome-Wide Association Studies that have dominated genetic research since the completion of the sequencing of the human genome in 2001 and have not yielded what was expected from the Human Genome Project (Paneth and Vermund 2018; Collins 1999).

Footnotes

[1] Fisher’s theorem states “The rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time.” According to Fisher, genetic diversity is analogous to disorder (entropy) and fitness is analogous to order. However, such analogies reflect a profound misunderstanding of thermodynamics. When thermodynamics is derived from the statistical physics of open systems, it becomes crystal clear that all systems evolve toward thermodynamic balance with their surroundings by minimizing free energy, equivalent to maximizing entropy, in the least time.

[2] The Modern Synthesis, framed in 1942 in a book by Julian Huxley, was the product of bringing together ideas that had been developing from the works of Mendel and Darwin on inheritance, natural selection, variation, and mutation with a mathematical basis See: https://en.wikipedia.org/wiki/Modern_synthesis_(20th_century) for a comprehensive account.

[3] Lenski explains the rationale thus: “a simple dynamical model with clonal interference (i.e., competition between lineages with different beneficial mutations) and diminishing-returns epistasis (i.e., beneficial mutations confer smaller advantages in more-fit than in less-fit backgrounds) generates a power-law relation” Lenski, R. E. (2017). "What is adaptation by natural selection? Perspectives of an experimental microbiologist." PLoS Genet **13**(4): e1006668.. This is

saying little more than “we can think of circumstances to mimic the power law trajectory”.

[4] We have chosen this source solely to illustrate what is typically believed, among geneticists, to be the proportion of common diseases attributable to genetic causes, not as a reference in other respects in which this book is flawed: see review by Joseph, J. (2022). "A Blueprint for Genetic Determinism." American Journal of Psychology **135**(4): 442-454.): The argument presented here that the gene is NOT Mendel’s unit of inheritance means that twin studies are null and void because the reason identical twins are so alike, is not their identical DNA sequences but the fact that they are derived from a single zygote.

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