

## Bacteria – The Last Stronghold of Lamarckism?

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### Abstract

French naturalist J.B. Lamarck is most commonly known for popularizing the theory that some traits acquired during the life of an organism can be inherited in his 1809 book. German biologist A. Weismann presented evidence in his 1891 book that acquired traits were not heritable in sexually reproducing animals. But so little was known about bacteria that they were considered to be the last stronghold of Lamarckism. The “fluctuation test” of S. Luria and M. Delbrück in 1943 seemed to confirm that Lamarckism in bacteria was indeed dead. This review, however, proposes that today bacteria may be viewed as the source from which much of our present knowledge of epigenetics, evolutionary developmental biology (evo-devo), and the induction or inheritance of acquired characters has grown.

French naturalist J.B. Lamarck (1744-1829) is best known for popularizing the ancient theory of the inheritance of adaptive acquired characteristics. He believed that unusual changes in an organism’s environment or activity can induce adaptive anatomical, physiological, or behavioral changes during the life of the individual that can then be transmitted to succeeding generations.

Acquired traits are phenotypes that develop during the lifetime of an individual in response to unusual environmental influences, rather than being determined by the genetic constitution of the individual. All acquired characters are not necessarily adaptive, but only adaptive traits are likely to persist over many generations in a population of organisms. Charles Darwin (1809-1882) did not know how phenotypic variations arose, and since Lamarckism was the only germane scientific theory of heredity then available, he subscribed to it.

By cutting off the tails of mice for several generations and breeding only from them, A. Weismann (1833-1914) reported in his 1891 book that the tail lengths of all the descendants grew to normal length (Weismann 1891). Many people assumed from these experiments that if characteristics acquired during the lifetime of individuals by such extreme measures had no heritable consequences, then the more subtle effects of natural environmental factors would also be ineffective in changing their hereditary endowment. Lamarckism thus fell into general disrespect as far as plants and animals are concerned. But not so with bacteria.

Even after the rediscovery of Mendelism in 1900, early bacteriologists thought that microbes had “soft heredity” that could be easily modified by changes in their environment. The “norm of reaction” is the phenotypic variability produced by a given genotype under the range of environmental conditions common to the natural habitat of the species under standard culture or experimental conditions. Pleiomorphism or “phenotypic plasticity” is a phenomenon in which a given genotype may develop

different states for a character or group of characters in different environments due to “genotype-environment interaction”. Some species of bacteria were discovered to have more than one form or shape (pleomorphic). For example, mycoplasmas are a group of bacteria with highly variable shape due to absence of a cell wall. Even genetically identical bacterial cells (clones) may develop different states for a character or group of characters in different environments.

We now know that some bacteria adaptively produce a protein (e.g., an inducible enzyme) only when needed, thus preventing wastage of energy required to produce a protein whose substrate is missing from the environment (Jacob and others 1960). For example, suppose that a bacterial cell is producing the sugar-digesting enzyme beta-galactosidase in the presence of its substrate (lactose) in the culture medium. It divides by fission to produce two genetically identical cells, one of which is transferred to lactose-free medium. The transferred cell may continue to contain the enzyme and/or its messenger RNA for one or more generations until the concentration of these products become sufficiently diluted or degraded to be no longer active. The term “perdurance” refers to a situation in which the phenotypic expression of a gene remains unchanged after the gene has been deleted or inactivated because of the long-lived nature of its product. Perdurance can thus be responsible for the inheritance of an acquired adaptive characteristic in bacteria.

### **The Beginning of Bacterial Genetics**

Bacterial genetics began with the publication of Salvatore Luria and Max Delbrück’s paper in the November 1943 issue of *Genetics*. Luria proclaimed that their “fluctuation test” removed bacteria from “the last stronghold of Larmarckism” (Fischer and Lipson 1988, p. 145). “Prior to the fluctuation test, the majority of bacteriologists favored the view that the environment directly influenced some or all of the cells in a population to become heritably adapted” (Adelberg 1960, p. x). At the same time, bacteriologists were still questioning if bacterial viruses (bacteriophage or phage) had any genes, and if phage did have genes it was not known to which class of biochemicals their hereditary material belonged. But in the next year, Avery, MacLeod and McCarty (1944) published a paper containing evidence that the substance transforming pneumococci from avirulence to virulence was deoxyribonucleic acid (DNA). A heat-inactivated (“killed”) strain S of pneumococcus bacteria was mixed with a live R strain of the same species. The virulent S strain forms “smooth” colonies on nutrient agar plates; the avirulent R strain forms “rough” colonies on nutrient agar plates. Avery recovered some S colonies from the admixture because DNA fragments released from the “dead” S cells had entered R cells and became integrated into the genomes of the host R cells, transforming them into virulent S phenotype.

The phenomenon of bacterial transduction was first described by N.D. Zinder and J. Lederberg (1952) using *Salmonella typhimurium* and phage P22. Transduction occurs when a bacteriophage infects a susceptible strain of bacteria and one or more bacterial genes become incorporated into the genome of the phage. When a recombinant phage infects another susceptible cell, the bacteria may incorporate into its own genome some

of the recombinant DNA from the previous host. The recipient cell may thus develop one or more new traits as a consequence of transduction.

In both bacterial transformation and transduction, recipient bacteria can “acquire” new traits by contact with factors in their extrinsic environment (raw DNA molecules in transformation; infection with recombinant phage in transduction). These newly acquired genes can (barring mutation) be stably transmitted from one asexual generation to another along with the rest of the genes in the recombinant cells. Thus, history shows that the fluctuation test did not remove bacteriology from “the last stronghold of Lamarckism” as Luria had claimed.

### **The Luria-Delbrück Fluctuation Test**

In a letter to Delbrück dated January 20, 1943, Luria outlined an experiment to determine if phage resistance in *E. coli* bacteria originates by spontaneous mutation or by contact with phage. [The phage reported in their November 1943 paper was called  $\alpha$ ; its name was later changed to T1]

“I thought that a clean cut experiment would be to find out how the fluctuations in the number of [phage-]resistants depend on the culture from which they came. That is: If I plate with ... [phage] ten samples of the *same* culture of [*E. coli* strain] B, I find numbers of resistants which fluctuate according to Poisson’s law. If I plate 10 samples of 10 *different* cultures of [*E. coli* strain] B, all containing the same amount of B, I find much larger fluctuations. If the resistants were produced on the plate, after contact with ... [phage], they should show the same fluctuations in both cases” (Fischer and Lipson 1988, p. 145).

The Luria-Delbrück fluctuation experiment (Luria and Delbrück 1943) compared the number of phage-resistant bacterial colonies observed in small individual cultures with those observed in samples from a large “bulk culture”. In one comparison, they set up 20 individual cultures of 0.2 ml each, and one 10 ml bulk culture, each containing an initial concentration of  $10^3$  phage-sensitive cells. These were grown to a concentration of  $2.8 \times 10^9$  cells/ml. Each of the individual cultures and ten 0.2 ml samples from the bulk culture were plated on separate plates covered uniformly with about  $10^{10}$  phage. Each plate thus received about the same number of bacterial cells ( $5.6 \times 10^8$ ). The number of phage-resistant colonies was counted after 12-16 hours of incubation. All ten of the samples from the bulk culture had about the same number of phage-resistant colonies (varying from 13-26; mean = 16.7; variance = 15; variance/mean = 0.9). Eleven of the 20 small cultures had no phage-resistant colonies; 9 of the other cultures had from 1 to 7 colonies (mean = 11.4; variance 694; variance/mean = 60.8). The Poisson distribution was formulated by the French mathematician and physicist Simeon D. Poisson (1781-1840). It is a function that assigns probabilities to the sequence of outcomes of observing no rare events of a specific type, one event, two events, and so forth. Events following a Poisson distribution are completely randomized. The Poisson is specified by the average number of rare events per observation; its mean and variance are equal; the variance/mean ratio thus equals 1.0. If phage-resistant bacteria are produced by exposure to phage, relatively small deviations should be seen in colony counts in all populations of the same size. On

the other hand, if phage-resistant cells are produced by spontaneous mutation at various times in the growth of cultures in the absence of phage, some cultures will experience an early mutation that replicates into large numbers of resistant cells by the time the experiment ends; later mutations will produce fewer phage-resistant colonies; most cultures will not have any mutations during this time; the number of phage-resistant colonies in different cultures is expected to vary (fluctuate) markedly. The results of this comparison tend to support the hypothesis that phage-resistance occurs by spontaneous random mutation rather than as an environmentally induced adaptive response to contact with phage.

Luria and Delbrück's 1943 evidence for the origin of phage-resistance in bacteria by mutation rather than by environmental induction "did for bacterial genetics what Mendel had done for general genetics – namely showed for the first time what kind of experimental arrangement, what kind of data analysis, and, above all, what kind of sophistication was needed for obtaining meaningful and unambiguous results ... their paper became the standard by which all later papers on bacterial genetics were to be measured" (Stent 1981). The fluctuation test not only provided evidence that resistance to phage T1 in *E. coli* bacteria were produced by spontaneous (random) mutation, but also provided a method for determining their mutation rates. In 1952, Lederberg and Lederberg used their "replica plating" technique to confirm the conclusions of Luria and Delbrück without exposing the cells to phage at any time.

### **Host Restriction and Modification**

"Host restriction and modification is a phenomenon in which a bacterium of a type X is able to distinguish a phage that has been grown in type X bacterium from one grown in a different type such as Y and is able to prevent the phage grown in Y from carrying out a successful infection" (Freifelder 1987). In his autobiography, Luria recalls how this "restriction and modification" phenomenon was discovered in 1952.

"While I was studying the breakup of DNA in phage-infected bacteria, I came upon a peculiar class of bacterial mutants. When infected by a certain phage the mutant cells were killed but seemed to produce no phage. This seemed mysterious. Was the phage lost, or did it produce some abnormal type of descendants? One day the test tube containing the phage-sensitive bacterial culture I was going to use happened to break. I have never been a very neat laboratory worker, and this time the breakage proved to be a lucky break. Rather than giving up my experiment, I got from my colleague Gio Bertani a sample of completely different bacteria, called *Shigella*, which we had reason to believe would work just as well. In fact they worked only too well. By the next day the mystery was cracked. My mutant bacteria had not failed to produce phage; they had produced a modified phage that refused to grow in its usual host, but grew perfectly well in Bertani's bacteria (which belonged to a different species). I had discovered the first instance of the phenomenon of *restriction* and *modification*. Phage that had grown in my mutant came out modified so that it could not multiply in any related bacteria, but could grow in different ones. In other words, the mutant allowed the phage to grow but modified it so that it could not grow except in *Shigella*. The *E. coli* bacteria restricted the modified phage" (Luria 1984).

Luria did not know the exact mechanism producing this phenomenon. In 1974, W.A. Arber proposed a restriction and modification model to explain it. According to this model, the DNA of a bacterium contains specific nucleotide sequences that can be recognized and cleaved by the restriction endonuclease carried by that cell. However, all cells that contain a restriction enzyme also contain a DNA methyl transferase enzyme that adds methyl groups ( $\text{CH}_3$ ) to these restriction sites. This chemical modification does not change the nucleotide sequence in DNA and thus is not a mutation. But it does protect the DNA of the host cell from its own restriction endonuclease. However, these nucleases can degrade unmethylated foreign DNA (bearing the target nucleotide sequence of the endonuclease) that might enter the host cell. The discovery of bacterial endonucleases eventually led to genetic engineering, genetic mapping, gene sequencing and other biotechnologies. The discovery of DNA methylation led to an understanding of a major mechanism for silencing specific genes during ontogeny and a basis for the epigenetic inheritance of acquired characteristics.

## **Epigenetics**

Both intrinsic and extrinsic environmental factors are now known to be involved in the differentiation of various cell types during embryological and postnatal development of an individual (ontogeny). “Epigenetics” is a branch of genetics that studies how phenotypic variants arise without changing the nucleotide sequence in DNA. The effects of epigenetic alterations to DNA or chromatin, though not often transmissible from one generation to the next, occasionally are inherited over one or more generations, and may be an underappreciated source of biological variation. “Inherited epigenetic variants can interact with their genetic counterparts to multiply by orders of magnitude the phenotypic variation available to natural selection, thereby expanding the mechanistic basis of evolutionary theoretical explanations and greatly increasing their plausibility as an account of life’s diversity” (Pigliucci 2006). The hybrid discipline of evolutionary developmental biology (“evo-devo”) studies, among other things, how organisms with identical genotypes may develop different phenotypes due to alteration of regulatory DNA sequences in response to different environmental factors.

Epigenetic methylation of specific DNA sequences near the transcription initiation region of genes has been shown to prevent transcription (gene inactivation) in a wide range of organisms including mice and humans. During embryological development from a zygote, cells differentiate in structure and function by programmed activation or inactivation of many genes at specific times, in specific anatomical locations, and with variable intensities. Regulatory DNA sequences (examples include attenuators, operators, and promoters) are not considered structural genes (coding for RNA molecules), but they are involved in regulating the expression of one or more structural genes. Mutation or methylation of these regulator regions can have heritable epigenetic effects. When both strands of a DNA molecule are methylated on opposite sites on the two strands and the molecule replicates, each of the double-stranded daughter molecules is initially methylated on only one strand, A methylase enzyme may recognize the mismatched (hemi-methylated) target site and add a methyl group to the unmethylated daughter

strand, thus reproducing the original fully methylated parental pattern (Saey 2009). Some methylation patterns persist from one generation to the next, thereby explaining the inheritance of some acquired traits. For example, male rats whose great grandfathers had been exposed to the fungicide vinclozolin, have lower fertility and higher risks of cancer than rats whose ancestors were not exposed to the fungicide (Young 2008). Evidence has been presented for the epigenetic inheritance of an adaptive anatomical trait in the water flea, *Daphnia* (Watters 2006). These crustaceans develop large, defensive spines when predators are nearby. Offspring of these armored parents also develop spines even in the absence of predators. In honeybees, diploid larvae normally fed royal jelly develop into reproductive queens; those not fed royal jelly develop into sterile worker bees. Silencing the gene for DNA methyltransferase in diploid larvae causes them to develop into reproductive queens who have never tasted royal jelly (Young 2008). Over 100 cases of transgenerational epigenetic inheritance in a wide range of organisms including bacteria, plants, and animals are documented in Jablonka and Raz (2009).

Some epigenetic effects are known to have implications for human health. Abnormal DNA methylation patterns may be involved in several human diseases. For example, methylation of a cancer producing oncogene may inactivate it, whereas, methylation of a tumor-suppressing gene may inactivate it, leading to cancer (Gibbs 2003). “Parental imprinting” is a phenomenon whereby the degree to which a gene is expressed depends upon the parent transmitting it. In humans, the same harmful gene mutation can produce either Prader-Willi or Angelman syndromes, depending on whether it is inherited from the mother or the father (Gibbs 2003; Jertle and Weidman 2007). The phenomenon may result from differing patterns of DNA methylation occurring during gametogenesis in the two sexes.

## **Conclusion**

History has shown that bacteria were not “the last stronghold of Lamarckism”, but rather the source from which much of our present knowledge of epigenetics, evolutionary developmental biology, and the induction or inheritance of acquired characters has grown.

## **References**

- Adelberg A. (Ed) 1960. *Papers on Bacterial Genetics*. Boston: Little, Brown and Company.
- Arber WL. 1974. DNA modification and restriction. *Pro. Nucleic Acid Res.* 14(1):1.
- Avery OT, MacLeod CM, McCarty M. 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcus types. *J Exp Med.* 79(2):137-158.
- Fischer EP, Lipson C. 1988. *Thinking About Science: Max Delbrück and the Origins of Molecular Biology*. New York: W.W. Norton & Company.

- Freifelder D. 1987. *Microbial Genetics*. Boston: Jones and Bartlett Publishers.
- Gibbs WW. 2003. The unseen genome: Beyond DNA. *Sci Amer*. 289:108-113.
- Jablonka E, Raz G. 2009. Transgenerational epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heredity and evolution. *Quart Rev Bio*. 84(2):131-176.
- Jacob F, Perrin D, Sanchez C, Monad J. 1960. The operon: A group of genes whose expression is coordinated by an operator. English translation in *Adelberg 1960*, pp. 395-397.
- Jertle RL, Weidman JR. 2007. Imprinted and more equal. *Amer Scientist*. 95:143-149.
- Lamarck JB. 1809. *Philosophie Zoologique*. English translation. Chicago: University of Chicago Press, 1984.
- Lederberg J, Lederberg EM. 1952. Replica plating and indirect selection of bacterial mutants. *J Bact*. 63:399-406.
- Luria SE. 1984. *A Slot Machine, A Broken Test Tube*. New York: Harper & Row, Publishers.
- Luria SE, Delbrück M. 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics*. 28:391-511.
- Pigliucci M. 2006. Have we solved Darwin's dilemma? *Amer. Scientist*. 94:272-274.
- Saey TH. 2009. Epigenetics: From islands to the shores. *Sci News*. 175:5-6.
- Stent GS. 1981. "Obituary: Max Delbrück" *Trends in Biochem Sci*. 6:iii-iv.
- Waters E. 2006. DNA is not destiny. *Discover*. 27(11):33.
- Weismann A. 1891. *Essays Upon Heredity and Kindred Biological Problems*. London: Oxford University Press.
- Young E. 2008. Strange inheritance. *New Scientist*. 199:29-33.
- Zinder ND, Lederberg J. 1952. Genetic exchange in *Salmonella*. *J Bact*. 64:679.