Sexuality and the Genetics of Bacteria

by
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Preface

"La seule différence entre les microbes et les espèces supérieures consisterait dans la rapidité des variations chez les virus, opposée à leur lenteur chez les grands êtres. Chaque culture d'un virus, n'eût-elle qu'une durée de vingt-quatre heures, représente des nombres immenses de générations successives tandis que, chez les êtres élevés, il faut, à l'accomplissement de tels nombres de générations, des milliers et des milliers d'années."

Louis Pasteur (1883)

Preface

Although the world of microorganisms was discovered three centuries ago, following the invention of the microscope, it was not until the work of Pasteur, however, that microbiology became an experimental science. In the space of a few years, man was amazed to discover the manifold roles of microorganisms, in the maintenance of life on earth and in the determination of his own destiny. The role played by the microbes in human affairs for a long time overshadowed their importance for the study of fundamental biological problems; during this period, microbiology remained isolated from the other biological sciences. Only a few decades ago was the emphasis on microorganisms as biological agents supplemented by the study of their own properties. The ensuing discoveries in microbial physiology largely contributed to establishing the fundamental unity of structure and function of all biological systems. When it was at last realized that microorganisms, and more especially bacteria, provide convenient material for the study of many problems in cellular biology, microbiology found itself established in a central and hitherto unanticipated position in the domain of biological sciences.

Among the basic disciplines of biology, it is with genetics that microbiology had the greatest difficulties in finding a common ground. For many years both geneticists and microbiologists tacitly agreed that the concepts and the methods of genetics did not apply to organisms devoid of sexual reproduction. It was only after genetics had been extended to microscopic fungi, and particularly after the fundamental work of Beadle and Tatum on the biochemical genetics of *Neurospora*, that an experimental approach to bacterial genetics was initiated. Within a few years, the study of mutations in bacteria and in bacterial viruses by Delbrück and Luria, together with the discovery of phenomena of genetic recombination in bacteria by Lederberg and Tatum and in bacterial viruses by Delbrück and by Hershey, demonstrated the fundamental unity of hereditary mechanisms throughout the living world. At the same time, the striking discovery by Avery, MacLeod, and McCarty that a bacterial character can be transformed by means of DNA extracted from a mutant provided the first information concerning the chemical basis of heredity.

Although these basic observations are all less than twenty years old, bacterial genetics has developed rapidly. This rapid development can be ascribed to several different causes. First of all is the simplicity, the extreme precision and the high resolving power of the methods available for the study of bacterial genetics. This has placed the fundamental problems of genetics within the competence of non-geneticists; some of the most remarkable contributions to microbial genetics have in fact been made by investigators trained in other branches of science, such as theoretical physics and physical chemistry. In the second place, with microorganisms it has proved relatively easy to relate genetic phenomena to the problems of structure and function of biologically important macromolecules. Finally, among microorganisms a variety of genetic systems and of genetic mechanisms are found which greatly facilitate genetic analysis. These factors have not only contributed to the very rapid progress of microbial genetics, but in addition, they have greatly extended knowledge about the nature and properties of the genetic material. As a result, bacterial and viral genetics have played a large part in the establishment of a new discipline, molecular biology, which occupies in the biological sciences the position that atomic physics has long occupied in the physical sciences.

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One of the most remarkable discoveries of microbial genetics is the revelation that the transfer of genetic information and genetic recombination do not occur uniquely through the classical mechanisms of sexual reproduction, but may also take place in a variety of other ways. In bacteria, for instance, there exist a number of types of genetic transfer which can cause a permanent modification of the hereditary properties of an organism. *Transformation*, discovered in *Pneumococcus* by Griffith and analyzed by Avery, McLeod, and McCarthy, involves the absorption by a recipient cell of DNA chemically extracted from a donor. *Transduction*, discovered in *Salmonella* by Zinder and Lederberg, is the transfer of genetic material from a donor to a recipient, by a bacteriophage acting as vector. *Bacterial conjugation*, which was discovered in *Escherichia coli* by Lederberg and Tatum, consists of the transfer of genetic material as a consequence of cellular contact between sexually differentiated bacteria. Of all phenomena of genetic transfer in bacteria, conjugation is the one which most closely resembles the sexual processes that exist in higher forms. It exhibits, however, certain peculiarities which relate it clearly to the other processes of genetic transfer in bacteria. *Lysogeny*, although differing somewhat from the other processes mentioned, is also a case of permanent modification of bacterial heredity. As shown by the work of

Lwoff and his group, it comprises an association, at the genetic level, between the genetic material of a bacterial virus and the bacterial host. Each of these genetic phenomena has its own characteristics and each of them presents its own particular advantages for the study of specific genetic problems.

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This monograph is an attempt to summarize our present knowledge concerning the process of sexual conjugation in bacteria and its use as a genetic system for the investigation of problems of cellular genetics. It also considers in detail the genetic aspects of lysogeny. The connection between these two subjects perhaps requires some explanation. Our work was initiated by an interest in the genetic determination of lysogeny; i.e., of the stable association which may become established between the genetic material of a virus and that of the host cell. The elucidation of this problem depended on a convenient method of genetic analysis; but, at the beginning of our investigation, knowledge of the mechanism operating in the process of bacterial conjugation was still very scanty. It fairly soon became apparent that, if progress in the genetic analysis of lysogeny was dependent upon a better understanding of the mechanism of bacterial conjugation, the information obtained in the study of lysogeny in turn helped considerably to unravel the mechanism of bacterial conjugation. The interdependence of progress in the two fields explains why the two subjects are treated together.

The way in which this book came to be written also needs to be explained. Originally, one of us had to publish a thesis which gave an account of some of the work done in our laboratory on bacterial conjugation. The need for a comprehensive source of information in French on bacterial genetics led us to prepare an enlarged and revised version of the original report.[1] When Academic Press kindly offered to publish an English translation of this monograph, we accepted with some reluctance, since we felt substantial changes would be necessary. Indeed, it soon became evident that the evolution of certain aspects of the subject necessitated extensive modification and development of the French version. Instead of rewriting the whole book, we chose to enlarge some of the existing chapters and to add new ones. The sporadic growth of the present book accordingly explains a certain lack of balance between the different parts, some discontinuity in the exposition, and some unavoidable repetitions. We ask the reader's indulgence for such unevenness. Had it not been written in so many stages, this monograph might have taken a

[1] E. L. Wollman and F. Jacob. La sexualité des bactéries. Masson ed. Paris, 1959.

rather different form. It is more likely, however, that it would have not been written at all!

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This monograph is composed of three parts. In the first part, the origin and early development of bacterial genetics are briefly outlined. The discovery of genetic recombination in bacteria and the characteristics of bacterial crosses with a low frequency of recombination, as originally described by Lederberg and Tatum, are then examined in more detail.

The second part is devoted to a detailed analysis of the process of sexual conjugation in bacteria. After describing the techniques and the characteristic features of crosses with a high frequency of recombination, we consider in turn the genetic analysis of lysogeny, the successive steps that can be defined in the processes of mating and genetic recombination, and the mechanism of genetic transfer during sexual conjugation. This analysis is followed by a comprehensive account of the different aspects of sexual conjugation in bacteria and of the genetic determination of sexual types. This section is more detailed than the other two. The different problems are analyzed in terms of the experimental results, and the theoretical conclusions are deduced step by step. This analytical approach will perhaps appear too technical and somewhat tedious to many readers. We have nonetheless adopted it, because we felt that it might prove useful for readers who are more directly interested in these problems, and particularly those who may be faced with the analysis of similar phenomena in groups of bacteria other than the Enterobacteriaceae.

In the third part, emphasis is placed on bacterial conjugation as a genetic system for the experimental attack on various problems of cellular genetics: specifically the structure of the genetic material, genetic recombination, and functional analysis. Certain problems raised by genetic studies with bacteria are also discussed: the genetic implications of incomplete fertilization, cellular regulation and gene action, the function of viruses as cellular genetic elements and, finally, the existence of other related cellular elements, or episomes, which are distinct from the normal constituents of the chromosomes.

Much of the experimental work described in this monograph has resulted from a close collaboration between the two authors in the Service de Physiologie microbienne at the Institut Pasteur. We are happy to have this opportunity of expressing our gratitude and our affection to Dr. André Lwoff and Dr. Jacques Monod, who have succeeded in creating around themselves a unique climate for research, characterized alike by

friendliness, by scientific enthusiasm, and by acute and pertinent criticism. We are particularly glad to express our sincere appreciation to Dr. J. Tréfouël, Director of the Institut Pasteur. We also wish to thank Mrs. Alberte Ungar for having undertaken the translation of a large part of the French monograph. Finally, we are greatly indebted to Dr. Robert Austrian and Dr. Roger Stanier for having helped with the revision of the final English version, and to Mrs. Gisèle Houzet, who has been indefatigable in preparing the successive drafts of this work.

Institut Pasteur
June, 1961

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PART I

Bacterial Genetics and the Discovery of Genetic Recombination in Bacteria

"Ce que nous devons viser, c'est moins de constater les ressemblances et les différences, que de retrouver les similitudes cachées sous les divergences apparentes."

HENRI POINCARÉ
(Science et Méthode)

CHAPTER 1

The Variability of Bacteria

Genetics originated from the study, in organisms which reproduce sexually, of hybridization between races or varieties of the same species, which differ in certain hereditary characters. The initial definition of the gene as the unit of recombination and segregation was only later extended to include also the unit of mutation and the unit of function. Finally, the integration of genetic data with cytological and cytochemical data led to the establishment and expansion of modern genetics.

For a long time, the methods of genetics seemed inapplicable to microorganisms in general, and to bacteria in particular. Bacteria reproduce vegetatively. Their small size made them unsuitable for cytological studies. Because of their low degree of organization, no distinction between soma and germen or between genotype and phenotype could be established. Consequently, it was long widely believed that the differences, both genetic and physiological, between higher organisms and microorganisms were of a fundamental nature, and that the laws valid for the former could not be applied to the latter. Bacteriologists and geneticists alike believed that bacteria did not have a differentiated genetic apparatus and that the mechanism of transmission of their hereditary characters was different from that revealed by genetic analysis in sexually reproducing organisms.

Because of their small size and rapid growth, bacteria can quickly produce an enormous population in a very small volume of culture medium. These conditions are highly favorable for the appearance of variant forms and for their study. The very characters that originally seemed to exclude genetic analysis of bacteria, in fact make them objects of choice for the study of variation, and it was from such studies that bacterial genetics was born.

Three phases in the evolution of ideas concerning the genetic constitution of bacteria can be broadly distinguished. The first one, covering the second half of the 19th century, was principally devoted to the description and isolation of bacterial species. During the second period, which extends from about 1900 to 1940, a large number of variations, affecting the most diverse properties of bacteria, were identified and described. Lastly, in the third period, starting about 1940, the facts concerning bacterial heredity were coordinated and analyzed in the light of classical genetic theory; and the simultaneous discovery of new phenomena, not explicable in terms of classical genetics, gave a further stimulus to bacterial genetics. It is not our intention to describe in detail this historical development; we shall content ourselves with outlining its main steps. Accounts of early work can be found in the following articles and reviews: Löhnis, 1921; Hadley, 1927; Brierley, 1929; Arkwright, 1930; Marchal, 1932; Bulloch, 1938. More recent work has likewise been frequently summarized (Dubos, 1945; Luria, 1947; Braun, 1947, 1953; Lederberg, 1948, 1949a; Tatum and Perkins, 1950; Catcheside, 1951; Kaplan, 1952; Wyss and Haas, 1953; Spiegelman and Landman, 1954; Zelle, 1955; Cavalli-Sforza, 1957; Ravin, 1958; Pontecorvo, 1958; Hartman and Goodgal, 1959; Beadle, 1960).

I. BACTERIAL SPECIES

Pasteur's investigations, starting with fermentations and continuing with infectious diseases, established both the microbial causation of these phenomena and the specificity of the causative agents. During the same period, a number of microscopists, the most eminent being F. Cohn (1872), observed and described a great many morphological varieties of microorganisms. The initial concern of bacteriologists was, accordingly, to grow and describe the microorganisms which they isolated and to establish the relation between their biological activity and their microscopic appearance. Because of the limited number of properties which could be used at this period to characterize bacteria, the proposed classifications were based mainly on morphological data. Some workers, such as Naegeli (1877), Büchner (1882), Zopf (1885), questioned the validity of a classification based on morphological criteria, and defended the theory of *pleomorphism.* According to this theory, bacteria may exhibit structures and even functions that are highly variable, with the result that the definition of bacterial *species* is illusory. The major contribution of F. Cohn (1879), and later of R. Koch (1881) and his school, was their defense of the opposing theory of *monomorphism,* which postulates the existence of bacterial categories that can be distinguished and classified on the basis of their morphological and physiological properties. As a result of progress in bacteriological technique, particularly the introduction of solid media, the isolation of bacteria in pure culture became easy. In the light of observations on large numbers of pure bacterial cultures, the theory of monomorphism rapidly won the acceptance of bacteriologists. One can thus regard this first period, which extends to the beginning of the 20th century, as having been mainly concerned with the descriptive study of bacteria and with the investigation of the diverse properties which could be used for the identification of species.[1]

General acceptance of the concept of monomorphism was the first essential step for the establishment, not only of bacterial genetics, but also of bacteriology as a science. However, like many other doctrines, monomorphism soon became dogmatic. Having performed a vital service by making possible the identification of bacterial species, it was illegitimately extended to assert the constancy of bacterial form and function, and to deny to bacterial species all possibility of variation.

II. BACTERIAL VARIATIONS

As the number of known bacterial species increased and as more and more of their specific properties were recognized, the variability of these properties became increasingly evident. Morphological characters, biochemical characters, antigenic properties, virulence—in fact, every one of the attributes that can be studied in bacteria—were found to be subject to variation. During the period between 1900 and 1940, the dogma of immutability was seriously challenged, and variability became recognized as one of the most important phenomena of microbiology.

With few exceptions, however, bacterial variations were the subject of observation rather than of experimentation. The most diverse kinds of variation were reported, and the proposed interpretations were not less numerous. Variations were described as being either slow or abrupt, reversible or irreversible, spontaneous or induced. For the most part, such distinctions depended on the conditions under which the variations were observed. The variation appeared slow or abrupt, continuous or discontinuous, depending on whether liquid cultures or isolated colonies were studied. If the variation was detected under normal cultural conditions (a morphological variation for instance), it appeared to be spontaneous; if its demonstration required the use of special methods (utilization of a sugar, for instance), it appeared to be induced.

Quite early, however, examples of sudden hereditary variations affecting easily recognizable characteristics were described. There were,

[1] The various systems proposed for the classification of bacteria are nothing more than arbitrary and empirical groupings designed to facilitate identification. This contention is particularly well documented by Van Niel (1946). The taxonomy of higher plants and animals can lay claim to being something more than an arbitrary arrangement of convenience because the fundamental taxonomic unit (the species) can be defined in objective scientific terms. A similar basis for defining bacterial species has not yet been discovered.