BACTERIAL GENETICS

BRAUN

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Preface

THE HISTORY of science is characterized by sudden spurts of progress which frequently occur following the merger of once separated disciplines. The field of bacterial genetics represents one of the most recent examples of such scientific cross-fertilization from which a subject with real hybrid vigor has evolved. The progress in this field during the last ten years has contributed not only significant information for geneticists, but what is perhaps even more important, it has resulted in the development of a branch of science that may take its place alongside biochemistry as necessary for understanding the nature and activity of microorganisms. Thus it has been recognized that a majority of problems in bacteriology and its allied fields, such as medicine, public health, bacterial metabolism and industrial fermentation, require an understanding of the nature and control of variation. This recognition of the growing importance of bacterial genetics is reflected in the remarkable increase of bacteriological publications that either deal with genetic problems or utilize the techniques developed by bacterial geneticists. In addition several universities have added formal courses on the subject to their curriculum.

Yet, despite the fact that bacterial genetics is gaining such importance in basic and applied research, it also has become evident that many students and professional workers without specialized training in genetics have had difficulties in understanding and following pertinent progress in this field. Apparently, there are two primary reasons for these difficulties: (1) the geneticists who work with bacteria have transferred many of their special terms into the field of bacterial genetics, and much of this language, as well as many of the underlying principles, are foreign to students of bacteriology; (2) the places of publication of fundamental data have been widely scattered, many of the important papers having appeared in journals that are read by very few bacteriologists.

This book has been written in an attempt to aid in overcoming these difficulties. It tries to present the more important findings and principles of bacterial genetics to those primarily trained, or being trained, in bacteriology, and it tries to provide a basis for better evaluation of the relationship between bacterial genetics and such diverse problems as pathogenesis, general laboratory techniques, bacterial physiology, epidemiology, therapy, and taxonomy. Beginning with a review of those general principles of genetics which are necessary for the understanding of bacterial genetics, the text endeavours to furnish a general account of the present status of bacterial genetics in a manner which, it is hoped, is elementary enough for the beginning student and sufficiently comprehensive to be of value for the research worker. But even though this book was written with the needs of bacteriologists in mind, it is hoped also that its contents will prove equally useful to students and research workers in the general field of genetics. The latter may find here not only a compilation of progress in an allied field but also information that should be of value to basic genetic problems.

In presenting this outline of bacterial genetics no attempt has been made to cover all the details of this rapidly expanding field and the space alloted to the various problems has been determined to a large extent by the apparent importance of the particular problem for general bacteriological questions. Thus the relatively extensive discussion of population changes should not be blamed on the author's preoccupation with such problems but on his conviction that these problems must be thoroughly understood and recognized by anyone connected with studies on bacteria. Throughout the book only representative examples of published data are cited and consequently many equally valuable contributions to the subject had to be omitted both in the text and in the literature references. In most instances, an effort has been made to cite those literature references which in turn will guide the reader to older publications.

I am greatly indebted to many colleagues and friends for their assistance in the preparation of this book. Many of them have contributed illustrations, including some previously unpublished material. The names of these contributors are indicated in the figure legends. I wish to express my collective thanks to them as well as

to the various publishers who gave permission for reproducing previously published figures and tables. I am particularly indebted to Drs. Emily Kelly, Edward Garber, Vernon Bryson and Mr. Leonard Mika, who read the manuscript in its entirety, and to Drs. Joshua Lederberg, Rollin Hotchkiss and Robert Austrian, who read certain chapters of the manuscript. All of them contributed valuable suggestions and editorial advice but, of course, they are in no way responsible for any remaining errors or omissions. The artistic skill of Mr. James Decker, who prepared all of the original drawings, and the diligence of Mr. Austin Hoffman, who typed the entire manuscript, were of invaluable help in the preparation of the book. I also wish to express my sincere appreciation to the staff of W. B. Saunders Co.; their interest and efficiency were of great help at all times. Finally, it is a real pleasure to acknowledge the interest of Dr. William Burrows without whose encouragement this book probably would still be an idea in the author's mind.

W.B.

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July, 1953

Contents

Chapter 6

Chapter 7

Chapter 8

Chapter 9

Chapter 10

Chapter 11

Chapter 12

Chapter 13

CHAPTER 1

Some General Genetic Principles

MORE THAN FIFTY YEARS OF GENETIC RESEARCH ON HIGHER organisms have demonstrated that, except for minor peculiarities in some species, the basic principles of genetics, the science of heredity, apply equally to all animals and plants. Information obtained in experimental work with the fruit fly, *Drosophila,* or with corn, therefore, can be and has been applied, often with immense economic gain, to such diverse problems as improvement of crops and livestock, increased penicillin yields, the discovery and understanding of the Rh factor, and the determination of disputed paternity. In recent years the area of applicability of general genetic principles has been broadened by the demonstration that the general mechanisms of inheritance in unicellular organisms, including bacteria, do not differ significantly from those established for higher organisms. Even though in most cases reproductive mechanisms appear to be less complex in bacteria than in higher organisms, all the available evidence indicates that the determinants of hereditary characteristics, their nature, sites, transmission, stability and the manner in which they control the processes leading to the actual development of the detectable characteristics, are similar in bacteria and in higher organisms. During its rapid development the field of bacterial genetics, therefore, was able to draw on the vast amount of existent general genetic information, the type of information which will be reviewed briefly in the following paragraphs.[1]

[1] This introductory survey is intended to acquaint the reader with genetic terminology and those basic genetic principles that are of interest to bacterial genetics. Within the confines of this survey it is impossible to do full justice to the wealth of data accumulated in genetic studies with higher organisms. For a more detailed account the reader is referred to one of the many textbooks of genetics. For example: Sinnot, E. W., Dunn, L. C., and Dobzhansky, Th., *Principles of Genetics,* McGraw-Hill Book Co., 1950; Srb, A. M. and Owen, R. D., *General Genetics,* W. H. Freeman & Co., San Francisco, 1952; Dunn, L. C. (Editor), *Genetics in the 20th Century,* The Macmillan Co., New York, 1951.

THE NATURE OF HEREDITARY CHARACTERISTICS

Genotype and phenotype

The old saying "like begets like" expresses the well-known fact that offspring or *progeny* usually mirror the general characteristics of their parents. This applies not only to higher organisms, where sexual processes are the rule rather than the exception, but also to asexually reproducing organisms. Hereditary traits thus are transmitted from generation to generation, and the manner in which they are transmitted has been the subject of intense experimental research, starting with the ingenious experiments on peas conducted by the Austrian monk, Gregor Mendel, in the 1860's. These studies, initially unnoticed, were independently rediscovered and extended by DeVries, Correns and Tschermak in 1900 and since then research has continued on various animals and plants in laboratories throughout the world. These extensive investigations on the principles of heredity and variation have demonstrated that what is transmitted from generation to generation is a set of determinants of as yet ill-defined chemical nature, controlling the reaction range of an individual or a cell. The sum total of these heritable determinants is called the *genotype*. Everyday experience shows us that despite similarity in genotype, as, for example, in identical twins or a group of purebred plants maintained under different environmental conditions, considerable differences may exist in appearance, i.e., the manner in which the inherited traits express themselves under the influence of the environment. What is inherited therefore is an ability to react in a specific way to specific environmental conditions. The sum total of the realized characteristics, in other words, the appearance of morphological characteristics as well as the manifestation of physiological processes in individuals, is called the *phenotype*. Phenotypic differences between organisms of similar genotype are referred to as *modifications*. Thus the interaction between hereditary determinants and environmental influences may cause divergent phenotypes despite the presence of identical genotypes; however, the genotype controls the potential range of such phenotypic differences. To choose a simple illustration let us consider the skin pigmentation of whites and Negroes. This difference in pigmentation is controlled by hereditary determinants *(genotypic differences)* yet the actual skin color will greatly depend on the extent of the individual's exposure to sunlight *(phenotypic differences)*. With prolonged exposure to sunlight, the skin of whites may become as dark as that of Negroes who have not been similarly exposed; yet after equally intense exposure the latter would manifest a far darker skin pigmentation than that ever possible for a white person (Fig. 1).

The foregoing example illustrates not only the potential plasticity existing in each organism or cell, but it also demonstrates that under certain environmental conditions the phenotypic expression of one genotype (the sum of inherited determinants) may be identical to that of an entirely different genotype. Such a phenomenon has been termed *phenocopy*.

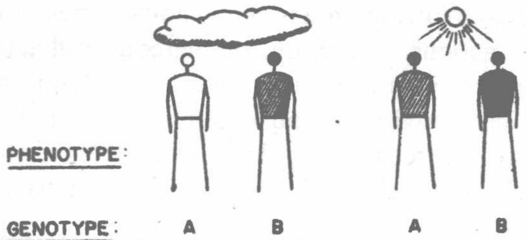

Fig. 1. Sketch illustrating the difference between phenotype and genotype (see text for a description of this example).

Even though environmental effects are capable of modifying the phenotype, as a rule such *modifications* persist only in the presence of the specific environmental condition and will not be transmitted to and inherited by the offspring. Cutting off the tails of mice for many generations does not reduce the length of tails in the progeny; neither does the exposure of flagellated bacteria to phenol, interfering with the development of flagella, cause the disappearance of flagella in the progeny when the latter are grown in phenol-free media. These and many other experimental studies have led to the conclusion that acquired characteristics probably are never inherited. This does not mean that the genotype remains entirely unaffected by the environment. As will be discussed subsequently, changes in the genotype occur occasionally in all organisms, and certain environmental agents are capable of enhancing the rate of such changes. In addition, the environment plays a most important role in promoting the selective survival of individuals with hereditary determinants that control characteristics best adapted to the existing environmental conditions.

It is now well established that practically all characteristics, both morphologic and physiologic, are under the direct or indirect control of hereditary determinants. In general, it is not the trait itself which is inherited, rather it is the potential to develop the particular trait under proper environmental conditions that is transmitted from generation to generation. Thus learning is not inherited, but the ability to learn is; cancer is not inherited but the tendency to develop certain types of cancer appears to be a hereditary trait. In the final analysis all hereditary characteristics are probably the end products of biochemical processes. This applies equally to physiological characteristics, morphological characteristics, and even to the ability to learn which depends on the development of the brain cells, a process involving an as yet unknown series of complex biosynthetic interactions. Therefore, the hereditary determinants may be said to control in a specific manner biosynthetic reactions and the biochemical activities of an organism. We shall discuss such mechanisms of control in more detail after a brief inquiry into the site of the hereditary determinants and their transmission.

THE SITE AND TRANSMISSION OF HEREDITARY DETERMINANTS

Chromosomes and genes

Most hereditary determinants are transmitted from generation to generation via nuclear components. Within the nucleus of cells there exist microscopically visible structures, usually filamentous in appearance, which are most easily observed when the nucleus is in the process of dividing. Due to their capacity to take up basic dyes these structures are called *chromosomes*. Chromosomes consist of nucleoproteins, i.e., proteins plus desoxyribose nucleic acid (DNA), and exhaustive experimental evidence has established that they are the carriers of factors or chemical complexes that control the inherited traits. These factors within the chromosomes are called genes; they are believed to represent macromolecules which are arranged in a linear fashion. While chromosomes are easily visible, the genes, or what is believed to be their site, have been only approximately localized. For example, the chromosomes of the salivary glands of insects are exceptionally large and, when stained with basic dyes, display a substructure consisting of bands with varying affinities for the stain. Certain alterations in hereditary traits have been found to be associated with changes in this visible substructure of salivary gland chromosomes (see also p. 14 and p. 186). These observations are merely one example of the correlation of cytological observations with the manifestations of genetically controlled processes, a correlation which has been of immense value in modern genetic research.

Chromosome numbers and mitosis

It was stressed above that as a rule the genotype, or the sum total of hereditary determinants, remains constant from generation to generation, as well as from cell to cell. Since the chromosomes have been recognized as the carriers of these determinants it must be expected that a mechanism exists whereby each cell receives from its parent cell a similar complement of chromosomal, and thus genic, material. Cytological observations have proven that this is the case, and that the manner in which chromosomes are distributed

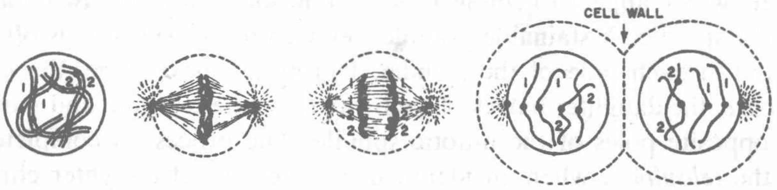

Fig. 2. Diagram of several stages of mitosis. Each cell in above sketch is diploid having 2 pairs of chromosomes. The dark dot on each chromosome, also shown in Figs. 3 and 57, indicates the centromere, a fixed structure which appears directly concerned with the movements of the chromosomes during division, leading the way on the spindle towards the poles.

from generation to generation, as well as from cell to cell, parallels the manner in which hereditary traits are transmitted. First of all it has been demonstrated that within a given species each nucleus contains a constant and characteristic number of chromosomes. For example, man has 24 pairs in each nucleus, *Drosophila melanogaster* 4 pairs, and certain roundworms have 1 pair. Considerable differences in size and shape are often seen between each pair of chromosomes (Fig. 3), but it is typical for sexually reproducing organisms that each chromosome is matched by a partner, or *homologous chromosome,* identical in size and shape and usually similar in its genic complement. Cells with pairs of chromosomes are called *diploid* cells. One member of each pair originates from the mother, the other from the father. During ordinary cell division equal shares of each chromosome are distributed to the daughter cells in a process known as *mitosis*. Figure 2 illustrates various stages of this