

A circular inset at the top left shows a purple-stained microorganism with internal structures. Another circular inset at the bottom left shows a dark field with numerous small, bright, circular spots, likely colonies of bacteria or yeasts.

CLASSIC PAPERS IN

GENETICS

Edited by

JAMES A. PETERS

1994
SH 42
DAG
C. A.

CLASSIC PAPERS IN GENETICS



EDITED BY

JAMES A. PETERS

ASSOCIATE PROFESSOR
SAN FERNANDO VALLEY STATE COLLEGE

PRENTICE-HALL, INC.

ENGLEWOOD CLIFFS, N. J.



PRENTICE-HALL BIOLOGICAL SCIENCE SERIES

Editors: William D. McElroy and Carl P. Swanson

PRENTICE-HALL BIOLOGICAL SCIENCE SERIES

Editors: William D. McElroy and Carl P. Swanson

CLASSIC PAPERS IN GENETICS, by James A. Peters

MILESTONES IN MICROBIOLOGY, by Thomas D. Brock

PRINCIPLES OF BIOLOGY, by Neal D. Buffaloe

A SYNTHESIS OF EVOLUTIONARY THEORY, by Herbert H. Ross

FOUNDATIONS OF MODERN BIOLOGY:

ADAPTATION, by Bruce Wallace and A. M. Srb

ANIMAL BEHAVIOR, by Vincent Dethier and Eliot Stellar

ANIMAL DIVERSITY, by Earl D. Hanson

ANIMAL GROWTH AND DEVELOPMENT, by Maurice Sussman

ANIMAL PHYSIOLOGY, by Knut Schmidt-Nielson

THE CELL, by Carl P. Swanson

CELLULAR PHYSIOLOGY AND BIOCHEMISTRY, by William D. McElroy

HEREDITY, by David M. Bonner

THE LIFE OF THE GREEN PLANT, by Arthur W. Galston

MAN IN NATURE, by Marston Bates

THE PLANT KINGDOM, by Harold C. Bold

Current printing (last digit):

16 15 14 13 12 11 10 9

© 1959, by PRENTICE-HALL, INC.

Englewood Cliffs, N. J.

All rights reserved. No part of this book may be reproduced in any form, by mimeograph or any other means, without permission in writing from the publishers. L.C. Cat. Card No.: 59-14106. Printed in the United States of America.

13517-C

Preface

Each paper in this collection has been selected for one or more reasons. It may have served to focus attention on a particular facet of genetics. It may illustrate well the impact the study of genetics has on biology or on social and racial relationships. It may have embodied a particular idea unique at the time of publication that has led to extensive research by other geneticists, in many cases still continuing today. It may provide a brilliant example of the use of the scientific method. It may furnish a clear-cut, concise illustration of incisive reasoning. One or two have the added virtue of having been written in an entertaining style.

In each case, they are evidence of work considered to constitute "classic" contributions to the science of biology. Taken as a unit, they have done much to give form and direction to genetic research. Their vitality is unimpaired by age, and their repeated citation in bibliographies of current literature or on seminar reading lists testifies that they are still important sources of information.

You should not expect, and will not find, any attempt by an author to "write down" to the level of his readers, for the primary concern is neither popularization nor condensation, but rather, adequate presentation. There is an assumption by the authors that the reader has some biological background. Lack of this background should not handicap anyone in following the development of the basic ideas. Most of the major steps in the development of the gene theory are here, and the nature of the material discussed by

each author was as new to biology at the time of writing as to any reader meeting it for the first time today.

This collection of papers served as the basis for a course in introductory biology taught for two years at Brown University. Many of these students had had no previous training in biology, but they demonstrated most satisfactorily that a neophyte in science can read, understand, and profit from a direct experience with the original literature of a particular field. Some guidance was necessary, and much was given in class. It is presented here in the form of an introduction to each paper. Little or no interpretation of the paper will be found in the introduction, however, for this interferes with the relationship between the author and the reader. All authors attempt to express their ideas clearly to the reader, and it is only fair to let them do so if they can. At the same time, the reader who follows an author's logic can feel that he has received his information from the primary source, and he is no longer dependent upon second hand interpretation of research.

It is my pleasure to acknowledge the permission granted by the publishers and authors to reproduce the papers in this volume. Citations to the original source are included with each paper. All of the journals are still being published except the Report of the Evolution Committee of the Royal Society, and they contain a continuing record of recent activities and researches. It would repay the reader to look over them occasionally to see what solutions have been offered to the many ques-

tions left unsolved by the authors included in this book.

It is also my pleasure to thank my students at Brown University for their work and study expended in reading,

discussing, and understanding these papers. A student who generates an interest in the subject he is studying is a joy forever, and I found myself blessed with a bountiful crop of them.

Note: All page references contained in the individual papers have been carried over into this volume from the original publications, for any value they might have for the researcher. Page numbers cited in the text do not refer to pages in this volume unless specifically so stated.

Table of Contents



Preface	iii
1865 • GREGOR MENDEL Experiments in Plant Hybridization	1
1903 • W. JOHANNSEN Heredity in Populations and Pure Lines	20
1903 • WALTER S. SUTTON The Chromosomes in Heredity	27
1905–1908 • W. BATESON and R. C. PUNNETT Experimental Studies in the Physiology of Heredity <i>Poultry, 44. Sweet Peas, 54.</i>	42
1908 • G. H. HARDY Mendelian Proportions in a Mixed Population	60
1910 • T. H. MORGAN Sex Limited Inheritance in Drosophila	63
1913 • A. H. STURTEVANT The Linear Arrangement of Six Sex-linked Factors in Drosophila, as Shown by their Mode of Association	67
1917 • SEWALL WRIGHT Color Inheritance in Mammals	78
1921 • L. C. DUNN Unit Character Variation in Rodents	92
1922 • H. J. MULLER Variation Due to Change in the Individual Gene	104
1925 • CALVIN B. BRIDGES Sex in Relation to Chromosomes and Genes	117
1925 • A. H. STURTEVANT The Effects of Unequal Crossing Over at the Bar Locus in Drosophila	124
1927 • H. J. MULLER Artificial Transmutation of the Gene	149

TABLE OF CONTENTS

1931 • HARRIET S. CREIGHTON AND BARBARA McCLINTOCK	
A Correlation of Cytological and Genetical Crossing-over in <i>Zea mays</i>	155
1933 • T. S. PAINTER	
A New Method for the Study of Chromosome Re-arrangements and Plotting of Chromosome Maps	161
1936 • CALVIN B. BRIDGES	
The Bar "Gene" a Duplication	163
1941 • G. W. BEADLE AND E. L. TATUM	
Genetic Control of Biochemical Reactions in <i>Neurospora</i>	166
1944 • O. T. AVERY, C. M. MACLEOD, AND MACLYN McCARTY	
Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types	173
1946 • J. LEDERBERG AND E. L. TATUM	
Gene Recombination in <i>Escherichia coli</i>	192
1947 • GENETICS CONFERENCE, COMMITTEE ON ATOMIC CASUALTIES, NATIONAL RESEARCH COUNCIL	
Genetic Effects of the Atomic Bombs in Hiroshima and Nagasaki	194
1950 • BARBARA McCLINTOCK	
The Origin and Behavior of Mutable Loci in Maize	199
1951 • N. H. HOROWITZ AND URS LEUPOLD	
Some Recent Studies Bearing on the One Gene-One Enzyme Hypothesis	209
1952 • N. D. ZINDER AND J. LEDERBERG	
Genetic Exchange in <i>Salmonella</i>	221
1953 • J. D. WATSON AND F. H. C. CRICK	
Molecular Structure of Nucleic Acids	241
1954 • L. J. STADLER	
The Gene	244
1954 • A. H. STURTEVANT	
Social Implications of the Genetics of Man	259
1955 • H. FRAENKEL-CONRAT AND R. C. WILLIAMS	
Reconstitution of Active Tobacco Mosaic Virus from its Inactive Protein and Nucleic Acid Components	264
1955 • SEYMOUR BENZER	
Fine Structure of a Genetic Region in Bacteriophage	271

Experiments in Plant-Hybridization

GREGOR MENDEL

This paper needs no introduction. It is the original classic paper on the theory of the gene, and the cornerstone of the science of genetics. The paper was translated from German into English by William Bateson, and has been reprinted several times.

Mendel's results are presented in a clear-cut and straightforward fashion, and his paper is fairly easy to read and understand. There have been comments made that Mendel was either very lucky or tampered with his data, because his results are almost miraculously close to perfect. Personally, I think both of these charges are arrant nonsense. Luck has little to do with results slowly accumulated over eight years' time. The results are the consequence of painstakingly careful attention to detail, followed by intelligent analysis of a mass of accumulated data. As to the second charge, that he might have arranged his data so as to shed the best possible light on his conclusions, I believe that the only way he might have manipulated his data is through omission of certain results that would have led to unnecessary complications. When Mendel specified that his experiments were to deal with "constantly differentiating characters" that occurred in pairs, he relieved himself of the necessity of considering some of the interrelationships that exist in genetic phenomena, and which will be discussed in the Bateson and Punnett papers (see pp. 44 and 54). Mendel probably knew of these interrelationships, because he tested many characters before selecting the seven pairs he used. The fact that he chose to utilize only those characteristics that fitted his concepts cannot be interpreted as an act of dishonesty on his part. As I see it, he recognized several of the basic concepts of heredity, and presented as much of his data as was necessary to validate those concepts.

I have not included the last few pages of Mendel's original paper, which dealt with experiments on hybrids of other species of plants, and with remarks on certain other questions of heredity. These paragraphs have little bearing on the principles Mendel proposed in this paper, and I have found from experience with my students that these pages serve primarily to confuse rather than to clarify.

INTRODUCTORY REMARKS¹

EXPERIENCE OF ARTIFICIAL FERTILISATION, such as is effected with ornamental plants in order to obtain new variations in colour, has led to the experiments which will here be discussed. The striking regularity with which the same hybrid forms always reappeared whenever fertilisation took place between the same species induced further experiments to be undertaken, the object of which was to follow up the developments of the hybrids in their progeny.

To this object numerous careful observers, such as Kölreuter, Gärtnner, Herbert, Lecoq, Wichura and others, have devoted a part of their lives with inexhaustible perseverance. Gärtnner especially, in his work "Die Bastardzeugung im Pflanzenreiche" (The Production of Hybrids in the Vegetable Kingdom), has recorded very valuable observations; and quite recently Wichura published the results of some profound investigations into the hybrids of the Willow. That, so far, no generally applicable law governing the formation and development of hybrids has been successfully formulated can hardly be wondered at by anyone who is acquainted with the extent of the task, and can appreciate the difficulties with which experiments of this class have to contend. A final decision can only be arrived at when we shall have before us the results of detailed experiments made on plants belonging to the most diverse orders.

Those who survey the work in this department will arrive at the convic-

¹ This translation was made by the Royal Horticultural Society of London, and is reprinted, by permission of the Council of the Society, with footnotes added and minor changes suggested by Professor W. Bateson, enclosed within []. The original paper was published in the *Verb. naturf. Ver. in Brunn, Abhandlungen*, iv. 1865, which appeared in 1866.

tion that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations.²

It requires indeed some courage to undertake a labour of such far-reaching extent; this appears, however, to be the only right way by which we can finally reach the solution of a question the importance of which cannot be overestimated in connection with the history of the evolution of organic forms.

The paper now presented records the results of such a detailed experiment. This experiment was practically confined to a small plant group, and is now, after eight years' pursuit, concluded in all essentials. Whether the plan upon which the separate experiments were conducted and carried out was the best suited to attain the desired end is left to the friendly decision of the reader.

SELECTION OF THE EXPERIMENTAL PLANTS

The value and utility of any experiment are determined by the fitness of the material to the purpose for which it is used, and thus in the case before us it cannot be immaterial what plants are subjected to experiment and in what manner such experiments are conducted.

The selection of the plant group which shall serve for experiments of this kind must be made with all pos-

² [It is to the clear conception of these three primary necessities that the whole success of Mendel's work is due. So far as I know this conception was absolutely new in his day.]

sible care if it be desired to avoid from the outset every risk of questionable results.

The experimental plants must necessarily—

1. Possess constant differentiating characters.

2. The hybrids of such plants must, during the flowering period, be protected from the influence of all foreign pollen, or be easily capable of such protection.

The hybrids and their offspring should suffer no marked disturbance in their fertility in the successive generations.

Accidental impregnation by foreign pollen, if it occurred during the experiments and were not recognized, would lead to entirely erroneous conclusions. Reduced fertility or entire sterility of certain forms, such as occurs in the offspring of many hybrids, would render the experiments very difficult or entirely frustrate them. In order to discover the relations in which the hybrid forms stand towards each other and also towards their progenitors it appears to be necessary that all members of the series developed in each successive generation should be, *without exception*, subjected to observation.

At the very outset special attention was devoted to the *Leguminosae* on account of their peculiar floral structure. Experiments which were made with several members of this family led to the result that the genus *Pisum* was found to possess the necessary qualifications.

Some thoroughly distinct forms of this genus possess characters which are constant, and easily and certainly recognizable, and when their hybrids are mutually crossed they yield perfectly fertile progeny. Furthermore, a disturbance through foreign pollen cannot easily occur, since the fertilising

organs are closely packed inside the keel and the anther bursts within the bud, so that the stigma becomes covered with pollen even before the flower opens. This circumstance is of especial importance. As additional advantages worth mentioning, there may be cited the easy culture of these plants in the open ground and in pots, and also their relatively short period of growth. Artificial fertilisation is certainly a somewhat elaborate process, but nearly always succeeds. For this purpose the bud is opened before it is perfectly developed, the keel is removed, and each stamen carefully extracted by means of forceps, after which the stigma can at once be dusted over with the foreign pollen.

In all, thirty-four more or less distinct varieties of Peas were obtained from several seedsmen and subjected to a two years' trial. In the case of one variety there were noticed, among a larger number of plants all alike, a few forms which were markedly different. These, however, did not vary in the following year, and agreed entirely with another variety obtained from the same seedsman; the seeds were therefore doubtless merely accidentally mixed. All the other varieties yielded perfectly constant and similar offspring; at any rate, no essential difference was observed during two trial years. For fertilisation twenty-two of these were selected and cultivated during the whole period of the experiments. They remained constant without any exception.

Their systematic classification is difficult and uncertain. If we adopt the strictest definition of a species, according to which only those individuals belong to a species which under precisely the same circumstances display precisely similar characters, no two of these varieties could be referred to one species. According to the opinion of

experts, however, the majority belong to the species *Pisum sativum*; while the rest are regarded and classed, some as sub-species of *P. sativum*, and some as independent species, such as *P. quadratum*, *P. saccharatum*, and *P. umbellatum*. The positions, however, which may be assigned to them in a classificatory system are quite immaterial for the purposes of the experiments in question. It has so far been found to be just as impossible to draw a sharp line between the hybrids of species and varieties as between species and varieties themselves.

DIVISION AND ARRANGEMENT OF THE EXPERIMENTS

If two plants which differ constantly in one or several characters be crossed, numerous experiments have demonstrated that the common characters are transmitted unchanged to the hybrids and their progeny; but each pair of differentiating characters, on the other hand, unite in the hybrid to form a new character, which in the progeny of the hybrid is usually variable. The object of the experiment was to observe these variations in the case of each pair of differentiating characters, and to deduce the law according to which they appear in the successive generations. The experiment resolves itself therefore into just as many separate experiments as there are constantly differentiating characters presented in the experimental plants.

The various forms of Peas selected for crossing showed differences in the length and colour of the stem; in the size and form of the leaves; in the position, colour, and size of the flowers; in the length of the flower stalk; in the colour, form, and size of the pods; in the form and size of the seeds; and in the colour of the seed-coats and of the albumen [cotyledons]. Some of the characters noted

do not permit of a sharp and certain separation, since the difference is of a "more or less" nature, which is often difficult to define. Such characters could not be utilised for the separate experiments; these could only be applied to characters which stand out clearly and definitely in the plants. Lastly, the result must show whether they, in their entirety, observe a regular behaviour in their hybrid unions, and whether from these facts any conclusion can be come to regarding those characters which possess a subordinate significance in the type.

The characters which were selected for experiment relate:

1. To the *difference in the form of the ripe seeds*. These are either round or roundish, the depressions, if any, occur on the surface, being always only shallow; or they are irregularly angular and deeply wrinkled (*P. quadratum*).

2. To the *difference in the colour of the seed albumen* (endosperm).³ The albumen of the ripe seeds is either pale yellow, bright yellow and orange coloured, or it possesses a more or less intense green tint. This difference of colour is easily seen in the seeds as [= if] their coats are transparent.

3. To the *difference in the colour of the seed-coat*. This is either white, with which character white flowers are constantly correlated; or it is grey, grey-brown, leather-brown, with or without violet spotting, in which case the colour of the standards is violet, that of the wings purple, and the stem in the axils of the leaves is of a reddish tint. The grey seed-coats become dark brown in boiling water.

4. To the *difference in the form of the ripe pods*. These are either simply

³ [Mendel uses the terms "albumen" and "endosperm" somewhat loosely to denote the cotyledons, containing food-material, within the seed.]

inflated, not contracted in places; or they are deeply constricted between the seeds and more or less wrinkled (*P. saccharatum*).

5. To the difference in the colour of the unripe pods. They are either light to dark green, or vividly yellow, in which colouring the stalks, leaf-veins, and calyx participate.⁴

6. To the difference in the position of the flowers. They are either axial, that is, distributed along the main stem; or they are terminal, that is, bunched at the top of the stem and arranged almost in a false umbel; in this case the upper part of the stem is more or less widened in section (*P. umbellatum*).⁵

7. To the difference in the length of the stem. The length of the stem⁶ is very various in some forms; it is, however, a constant character for each, in so far that healthy plants, grown in the same soil, are only subject to unimportant variations in this character.

In experiments with this character, in order to be able to discriminate with certainty, the long axis of 6 to 7 ft. was always crossed with the short one of $\frac{3}{4}$ ft. to $1\frac{1}{2}$ ft.

Each two of the differentiating characters enumerated above were united by cross-fertilisation. There were made for the

⁴ One species possesses a beautifully brownish-red coloured pod, which when ripening turns to violet and blue. Trials with this character were only begun last year. [Of these further experiments it seems no account was published. Correns has since worked with such a variety.]

⁵ [This is often called the Mummy Pea. It shows slight fasciation. The form I know has white standard and salmon-red wings.]

⁶ [In my account of these experiments (*R.H.S. Journal*, vol. xxv. p. 54) I misunderstood this paragraph and took "axis" to mean the *floral axis*, instead of the main axis of the plant. The unit of measurement, being indicated in the original by a dash ('), I carelessly took to have been an *inch*, but the translation here given is evidently correct.]

1st trial	60	fertilisations	on 15	plants.
2nd "	58	"	"	10 "
3rd "	35	"	"	10 "
4th "	40	"	"	10 "
5th "	23	"	"	5 "
6th "	34	"	"	10 "
7th "	37	"	"	10 "

From a larger number of plants of the same variety only the most vigorous were chosen for fertilisation. Weakly plants always afford uncertain results, because even in the first generation of hybrids, and still more so in the subsequent ones, many of the offspring either entirely fail to flower or only form a few and inferior seeds.

Furthermore, in all the experiments reciprocal crossings were effected in such a way that each of the two varieties which in one set of fertilisation served as seed-bearer in the other set was used as the pollen plant.

The plants were grown in garden beds, a few also in pots, and were maintained in their naturally upright position by means of sticks, branches of trees, and strings stretched between. For each experiment a number of pot plants were placed during the blooming period in a greenhouse, to serve as control plants for the main experiment in the open as regards possible disturbance by insects. Among the insects⁷ which visit Peas the beetle *Bruchus pisi* might be detrimental to the experiments should it appear in numbers. The female of this species is known to lay the eggs in the flower, and in so doing opens the keel; upon the tarsi of one specimen, which was caught in a flower, some pollen grains could clearly be seen under a lens. Mention must also be made of a circumstance which possibly might lead

⁷ [It is somewhat surprising that no mention is made of Thrips, which swarm in Pea flowers. I had come to the conclusion that this is a real source of error and I see Laxton held the same opinion.]

to the introduction of foreign pollen. It occurs, for instance, in some rare cases that certain parts of an otherwise quite normally developed flower wither, resulting in a partial exposure of the fertilising organs. A defective development of the keel has also been observed, owing to which the stigma and anthers remained partially uncovered.⁸ It also sometimes happens that the pollen does not reach full perfection. In this event there occurs a gradual lengthening of the pistil during the blooming period, until the stigmatic tip protrudes at the point of the keel. This remarkable appearance has also been observed in hybrids of *Phaseolus* and *Lathyrus*.

The risk of false impregnation by foreign pollen is, however, a very slight one with *Pisum*, and is quite incapable of disturbing the general result. Among more than 10,000 plants which were carefully examined there were only a very few cases where an indubitable false impregnation had occurred. Since in the greenhouse such a case was never remarked, it may well be supposed that *Bruchus pisi*, and possibly also the described abnormalities in the floral structure, were to blame.

[F₁] THE FORMS OF THE HYBRIDS⁹

Experiments which in previous years were made with ornamental plants have already afforded evidence that the hybrids, as a rule, are not exactly intermediate between the parental species. With some of the more striking characters, those, for instance, which relate to the form and size of the leaves, the pubescence of the several parts, &c., the intermediate, in-

deed, is nearly always to be seen; in other cases, however, one of the two parental characters is so preponderant that it is difficult, or quite impossible, to detect the other in the hybrid.

This is precisely the case with the Pea hybrids. In the case of each of the seven crosses the hybrid-character resembles¹⁰ that of one of the parental forms so closely that the other either escapes observation completely or cannot be detected with certainty. This circumstance is of great importance in the determination and classification of the forms under which the offspring of the hybrids appear. Henceforth in this paper those characters which are transmitted entire, or almost unchanged in the hybridisation, and therefore in themselves constitute the characters of the hybrid, are termed the *dominant*, and those which become latent in the process *recessive*. The expression "recessive" has been chosen because the characters thereby designated withdraw or entirely disappear in the hybrids, but nevertheless reappear unchanged in their progeny, as will be demonstrated later on.

It was furthermore shown by the whole of the experiments that it is perfectly immaterial whether the dominant character belongs to the seed-bearer or to the pollen-parent; the form of the hybrid remains identical in both cases. This interesting fact was also emphasised by Gärtner, with the remark that even the most practised expert is not in a position to determine in a hybrid which of the two parental species was the seed or the pollen plant.¹¹

Of the differentiating characters

⁸ [This also happens in Sweet Peas.]

⁹ [Mendel throughout speaks of his cross-bred Peas as "hybrids," a term which many restrict to the offspring of two distinct species. He, as he explains, held this to be only a question of degree.]

¹⁰ [Note that Mendel, with true penetration, avoids speaking of the hybrid-character as "transmitted" by either parent, thus escaping the error pervading the older views of heredity.]

¹¹ [Gärtner, p. 223.]

which were used in the experiments the following are dominant:

1. The round or roundish form of the seed with or without shallow depressions.

2. The yellow colouring of the seed albumen [cotyledons].

3. The grey, grey-brown, or leather-brown colour of the seed-coat, in association with violet-red blossoms and reddish spots in the leaf axils.

4. The simply inflated form of the pod.

5. The green colouring of the unripe pod in association with the same colour in the stems, the leaf-veins and the calyx.

6. The distribution of the flowers along the stem.

7. The greater length of stem.

With regard to this last character it must be stated that the longer of the two parental stems is usually exceeded by the hybrid, a fact which is possibly only attributable to the greater luxuriance which appears in all parts of plants when stems of very different length are crossed. Thus, for instance, in repeated experiments, stems of 1 ft. and 6 ft. in length yielded without exception hybrids which varied in length between 6 ft. and 7½ ft.

The hybrid seeds in the experiments with seed-coat are often more spotted, and the spots sometimes coalesce into small bluish-violet patches. The spotting also frequently appears even when it is absent as a parental character.¹²

The hybrid forms of the seed-shape and of the albumen [colour] are developed immediately after the artificial fertilisation by the mere influence of the foreign pollen. They can, therefore, be observed even in the first year of experiment, whilst all the other characters naturally only appear in the

following year in such plants as have been raised from the crossed seed.

[*F*₂] THE GENERATION [BRED] FROM THE HYBRIDS

In this generation there reappear, together with the dominant characters, also the recessive ones with their peculiarities fully developed, and this occurs in the definitely expressed average proportion of three to one, so that among each four plants of this generation three display the dominant character and one the recessive. This relates without exception to all the characters which were investigated in the experiments. The angular wrinkled form of the seed, the green colour of the albumen, the white colour of the seed-coats and the flowers, the constrictions of the pods, the yellow colour of the unripe pod, of the stalk, of the calyx, and of the leaf venation, the umbel-like form of the inflorescence, and the dwarfed stem, all reappear in the numerical proportion given, without any essential alteration. *Transitional forms were not observed in any experiment.*

Since the hybrids resulting from reciprocal crosses are formed alike and present no appreciable difference in their subsequent development, consequently the results [of the reciprocal crosses] can be reckoned together in each experiment. The relative numbers which were obtained for each pair of differentiating characters are as follows:

Expt. 1. Form of seed.—From 253 hybrids 7,324 seeds were obtained in the second trial year. Among them were 5,474 round or roundish ones and 1,850 angular wrinkled ones. Therefrom the ratio 2.96 to 1 is deduced.

Expt. 2. Colour of albumen.—258 plants yielded 8,023 seeds, 6,022 yellow, and 2,001 green; their ratio, therefore, is as 3.01 to 1.

In these two experiments each pod

¹² [This refers to the coats of the seeds borne by *F*₁ plants.]

yielded usually both kinds of seeds. In well-developed pods which contained on the average six to nine seeds, it often happened that all the seeds were round (Expt. 1) or all yellow (Expt. 2); on the other hand there were never observed more than five wrinkled or five green ones in one pod. It appears to make no difference whether the pods are developed early or later in the hybrid or whether they spring from the main axis or from a lateral one. In some few plants only a few seeds developed in the first formed pods, and these possessed exclusively one of the two characters, but in the subsequently developed pods the normal proportions were maintained nevertheless.

As in separate pods, so did the distribution of the characters vary in separate plants. By way of illustration the first ten individuals from both series of experiments may serve.

Plants	EXPERIMENT 1.		EXPERIMENT 2.			
	Form of Seed.	Color of Albumen.	Round	Angular	Yellow	Green
1	45		12		25	11
2	27		8		32	7
3	24		7		14	5
4	19		16		70	27
5	32		11		24	13
6	26		6		20	6
7	88		24		32	13
8	22		10		44	9
9	28		6		50	14
10	25		7		44	18

As extremes in the distribution of the two seed characters in one plant, there were observed in Expt. 1 an instance of 43 round and only 2 angular, and another of 14 round and 15 angular seeds. In Expt. 2 there was a case of 32 yellow and only 1 green seed, but also one of 20 yellow and 19 green.

These two experiments are important for the determination of the average ratios, because with a smaller num-

ber of experimental plants they show that very considerable fluctuations may occur. In counting the seeds, also, especially in Expt. 2, some care is requisite, since in some of the seeds of many plants the green colour of the albumen is less developed, and at first may be easily overlooked. The cause of this partial disappearance of the green colouring has no connection with the hybrid-character of the plants, as it likewise occurs in the parental variety. This peculiarity [bleaching] is also confined to the individual and is not inherited by the offspring. In luxuriant plants this appearance was frequently noted. Seeds which are damaged by insects during their development often vary in colour and form, but, with a little practice in sorting, errors are easily avoided. It is almost superfluous to mention that the pods must remain on the plants until they are thoroughly ripened and have become dried, since it is only then that the shape and colour of the seed are fully developed.

Expt. 3. Colour of the seed-coats.—Among 929 plants 705 bore violet-red flowers and grey-brown seed-coats; 224 had white flowers and white seed-coats, giving the proportion 3.15 to 1.

Expt. 4. Form of pods.—Of 1,181 plants 882 had them simply inflated, and in 299 they were constricted. Resulting ratio, 2.95 to 1.

Expt. 5. Colour of the unripe pods.—The number of trial plants was 580, of which 428 had green pods and 152 yellow ones. Consequently these stand in the ratio 2.82 to 1.

Expt. 6. Position of flowers.—Among 858 cases 651 had inflorescences axial and 207 terminal. Ratio, 3.14 to 1.

Expt. 7. Length of stem.—Out of 1,064 plants, in 787 cases the stem was long, and in 277 short. Hence a mutual ratio of 2.84 to 1. In this experiment the dwarfed plants were carefully

lifted and transferred to a special bed. This precaution was necessary, as otherwise they would have perished through being overgrown by their tall relatives. Even in their quite young state they can be easily picked out by their compact growth and thick dark-green foliage.¹³

If now the results of the whole of the experiments be brought together, there is found, as between the number of forms with the dominant and recessive characters, an average ratio of 2.98 to 1, or 3 to 1.

The dominant character can have here a *double signification*—viz. that of a parental character, or a hybrid-character.¹⁴ In which of the two significations it appears in each separate case can only be determined by the following generation. As a parental character it must pass over unchanged to the whole of the offspring; as a hybrid-character, on the other hand, it must maintain the same behaviour as in the first generation [F_2].

[F_3] THE SECOND GENERATION [BRED] FROM THE HYBRIDS

Those forms which in the first generation [F_2] exhibit the recessive character do not further vary in the second generation [F_3] as regards this character; they remain constant in their offspring.

It is otherwise with those which possess the dominant character in the first generation [bred from the hybrids]. Of these *two-thirds* yield offspring which display the dominant and recessive characters in the proportion of 3 to 1, and thereby show exactly

¹³ [This is true also of the dwarf or "Cupid" Sweet Peas.]

¹⁴ [This paragraph presents the view of the hybrid-character as something incidental to the hybrid, and not "transmitted" to it—a true and fundamental conception here expressed probably for the first time.]

the same ratio as the hybrid forms, while only *one-third* remains with the dominant character constant.

The separate experiments yielded the following results:

Expt. 1. Among 565 plants which were raised from round seeds of the first generation, 193 yielded round seeds only, and remained therefore constant in this character; 372, however, gave both round and wrinkled seeds, in the proportion of 3 to 1. The number of the hybrids, therefore, as compared with the constants is 1.93 to 1.

Expt. 2. Of 519 plants which were raised from seeds whose albumen was of yellow colour in the first generation, 166 yielded exclusively yellow, while 353 yielded yellow and green seeds in the proportion of 3 to 1. There resulted, therefore, a division into hybrid and constant forms in the proportion of 2.13 to 1.

For each separate trial in the following experiments 100 plants were selected which displayed the dominant character in the first generation, and in order to ascertain the significance of this, ten seeds of each were cultivated.

Expt. 3. The offspring of 36 plants yielded exclusively grey-brown seed-coats, while of the offspring of 64 plants some had grey-brown and some had white.

Expt. 4. The offspring of 29 plants had only simply inflated pods; of the offspring of 71, on the other hand, some had inflated and some constricted.

Expt. 5. The offspring of 40 plants had only green pods; of the offspring of 60 plants some had green, some yellow ones.

Expt. 6. The offspring of 33 plants had only axial flowers; of the offspring of 67, on the other hand, some had axial and some terminal flowers.

Expt. 7. The offspring of 28 plants

inherited the long axis, and those of 72 plants some the long and some the short axis.

In each of these experiments a certain number of the plants came constant with the dominant character. For the determination of the proportion in which the separation of the forms with the constantly persistent character results, the two first experiments are of especial importance, since in these a larger number of plants can be compared. The ratios 1.93 to 1 and 2.13 to 1 gave together almost exactly the average ratio of 2 to 1. The sixth experiment gave a quite concordant result; in the others the ratio varies more or less, as was only to be expected in view of the smaller number of 100 trial plants. Experiment 5, which shows the greatest departure, was repeated, and then, in lieu of the ratio of 60 and 40, that of 65 and 35 resulted. *The average ratio of 2 to 1 appears, therefore, as fixed with certainty.* It is therefore demonstrated that, of those forms which possess the dominant character in the first generation, two-thirds have the hybrid-character, while one-third remains constant with the dominant character.

The ratio of 3 to 1, in accordance with which the distribution of the dominant and recessive characters results in the first generation, resolves itself therefore in all experiments into the ratio of 2:1:1 if the dominant character be differentiated according to its significance as a hybrid-character or as a parental one. Since the members of the first generation [F_2] spring directly from the seed of the hybrids [F_1], it is now clear that the hybrids form seeds having one or other of the two differentiating characters, and of these one-half develop again the hybrid form, while the other half yield plants which remain constant and receive the dominant or the recessive

characters [respectively] in equal numbers.

THE SUBSEQUENT GENERATIONS [BRED] FROM THE HYBRIDS

The proportions in which the descendants of the hybrids develop and split up in the first and second generations presumably hold good for all subsequent progeny. Experiments 1 and 2 have already been carried through six generations, 3 and 7 through five, and 4, 5, and 6 through four, these experiments being continued from the third generation with a small number of plants, and no departure from the rule has been perceptible. The offspring of the hybrids separated in each generation in the ratio of 2:1:1 into hybrids and constant forms.

If A be taken as denoting one of the two constant characters, for instance the dominant, a , the recessive, and Aa the hybrid form in which both are conjoined, the expression

$$A + 2Aa + a$$

shows the terms in the series for the progeny of the hybrids of two differentiating characters.

The observation made by Gartner, Kolreuter, and others, that hybrids are inclined to revert to the parental forms, is also confirmed by the experiments described. It is seen that the number of the hybrids which arise from one fertilisation, as compared with the number of forms which become constant, and their progeny from generation to generation, is continually diminishing, but that nevertheless they could not entirely disappear. If an average equality of fertility in all plants in all generations be assumed, and if, furthermore, each hybrid forms seed of which one-half yields hybrids again, while the other half is constant to both characters in equal proportions, the ratio of numbers for the offspring in

each generation is seen by the following summary, in which *A* and *a* denote again the two parental characters, and *Aa* the hybrid forms. For brevity's sake it may be assumed that each plant in each generation furnishes only 4 seeds.

Genera- tion				RATIOS
	<i>A</i>	<i>Aa</i>	<i>a</i>	<i>A</i> : <i>Aa</i> : <i>a</i>
1	1	2	1	1 : 2 : 1
2	6	4	6	3 : 2 : 3
3	28	8	28	7 : 2 : 7
4	120	16	120	15 : 2 : 15
5	496	32	496	31 : 2 : 31
<i>n</i>				$2^n - 1 : 2 : 2^n - 1$

In the tenth generation, for instance, $2^n - 1 = 1023$. There result, therefore, in each 2,048 plants which arise in this generation 1,023 with the constant dominant character, 1,023 with the recessive character, and only two hybrids.

THE OFFSPRING OF HYBRIDS IN WHICH SEVERAL DIFFERENTIATING CHARACTERS ARE ASSOCIATED

In the experiments above described plants were used which differed only in one essential character.¹⁵ The next task consisted in ascertaining whether the law of development discovered in these applied to each pair of differentiating characters when several diverse characters are united in the hybrid by crossing. As regards the form of the hybrids in these cases, the

¹⁵ [This statement of Mendel's in the light of present knowledge is open to some misconception. Though his work makes it evident that such varieties may exist, it is very unlikely that Mendel could have had seven pairs of varieties such that the members of each pair differed from each other in *only* one considerable character (*wesentliches Merkmal*). The point is probably of little theoretical or practical consequence, but a rather heavy stress is thrown on "*wesentlich*."]

experiments showed throughout that this invariably more nearly approaches to that one of the two parental plants which possesses the greater number of dominant characters. If, for instance, the seed plant has a short stem, terminal white flowers, and simply inflated pods; the pollen plant, on the other hand, a long stem, violet-red flowers distributed along the stem, and constricted pods; the hybrid resembles the seed parent only in the form of the pod; in the other characters it agrees with the pollen parent. Should one of the two parental types possess only dominant characters, then the hybrid is scarcely or not at all distinguishable from it.

Two experiments were made with a considerable number of plants. In the first experiment the parental plants differed in the form of the seed and in the colour of the albumen; in the second in the form of the seed, in the colour of the albumen, and in the colour of the seed-coats. Experiments with seed characters give the result in the simplest and most certain way.

In order to facilitate study of the data in these experiments, the different characters of the seed plant will be indicated by *A*, *B*, *C*, those of the pollen plant by *a*, *b*, *c*, and the hybrid forms of the characters by *Aa*, *Bb*, and *Cc*.

Expt. 1.—*AB*, seed parents;
A, form round;
B, albumen yellow.
ab, pollen parents;
a, form wrinkled;
b, albumen green.

The fertilised seeds appeared round and yellow like those of the seed parents. The plants raised therefrom yielded seeds of four sorts, which frequently presented themselves in one pod. In all, 556 seeds were yielded by 15 plants, and of these there were:

315 round and yellow,
101 wrinkled and yellow,
108 round and green,
32 wrinkled and green.

All were sown the following year. Eleven of the round yellow seeds did not yield plants, and three plants did not form seeds. Among the rest:

38 had round yellow seeds	<i>AB</i>
65 round yellow and green seeds	<i>Ab</i>
60 round yellow and wrinkled yellow seeds	<i>aB</i>
138 round yellow and green, wrinkled yellow and green seeds	<i>Aab</i>

From the wrinkled yellow seeds 96 resulting plants bore seed, of which:

28 had only wrinkled yellow seeds	<i>ab</i>
68 wrinkled yellow and green seeds	<i>aBb</i> .

From 108 round green seeds 102 resulting plants fruited, of which:

35 had only round green seeds	<i>Ab</i>
67 round and wrinkled green seeds	<i>Aab</i> .

The wrinkled green seeds yielded 30 plants which bore seeds all of like character; they remained constant *ab*.

The offspring of the hybrids appeared therefore under nine different forms, some of them in very unequal numbers. When these are collected and co-ordinated we find:

38	plants with the sign	<i>AB</i>
35	" "	<i>Ab</i>
28	" "	<i>aB</i>
30	" "	<i>ab</i>
65	" "	<i>Abb</i>
68	" "	<i>aBb</i>
60	" "	<i>AaB</i>
67	" "	<i>Aab</i>
138	" "	<i>AaBb</i> .

The whole of the forms may be classed into three essentially different groups. The first includes those with the signs *AB*, *Ab*, *aB*, and *ab*: they

possess only constant characters and do not vary again in the next generation. Each of these forms is represented on the average thirty-three times. The second group includes the signs *Abb*, *aBb*, *AaB*, *Aab*: these are constant in one character and hybrid in another, and vary in the next generation only as regards the hybrid-character. Each of these appears on an average sixty-five times. The form *Aab* occurs 138 times: it is hybrid in both characters, and behaves exactly as do the hybrids from which it is derived.

If the numbers in which the forms belonging to these classes appear be compared, the ratios of 1, 2, 4 are unmistakably evident. The numbers 33, 65, 138 present very fair approximations to the ratio numbers of 33, 66, 132.

The developmental series consists, therefore, of nine classes, of which four appear therein always once and are constant in both characters; the forms *AB*, *ab*, resemble the parental forms, the two other present combinations between the conjoined characters *A*, *a*, *B*, *b*, which combinations are likewise possibly constant. Four classes appear always twice, and are constant in one character and hybrid in the other. One class appears four times, and is hybrid in both characters. Consequently the offspring of the hybrids, if two kinds of differentiating characters are combined therein, are represented by the expression

$$AB + Ab + aB + ab + 2Abb + 2aBb + 2AaB + 2Aab + 4AaBb.$$

This expression is indisputably a combination series in which the two expressions for the characters *A* and *a*, *B* and *b* are combined. We arrive at the full number of the classes of the series by the combination of the expressions:

$$\begin{aligned}A + 2Aa + a \\B + 2Bb + b.\end{aligned}$$

Expt. 2.

ABC, seed parents;*A*, form round;*B*, albumen yellow;*C*, seed-coat grey-brown.*abc*, pollen parents;*a*, form wrinkled;*b*, albumen green;*c*, seed-coat white.

This experiment was made in precisely the same way as the previous one. Among all the experiments it demanded the most time and trouble. From 24 hybrids 687 seeds were obtained in all: these were all either spotted, grey-brown or grey-green, round or wrinkled.¹⁶ From these in the following year 639 plants fruited, and, as further investigation showed, there were among them:

	8 plants <i>ABC</i>	22 plants <i>ABCc</i>	45 plants <i>ABbCc</i>
14	" <i>ABC</i>	17 " <i>AbCc</i>	36 " <i>aBbCc</i>
9	" <i>AbC</i>	25 " <i>abCc</i>	38 " <i>AabCc</i>
11	" <i>Abc</i>	20 " <i>abCc</i>	40 " <i>AabCc</i>
8	" <i>aBC</i>	15 " <i>ABbC</i>	49 " <i>AabBc</i>
10	" <i>aBc</i>	18 " <i>ABbc</i>	48 " <i>AAbbc</i>
10	" <i>abC</i>	19 " <i>aBbC</i>	
7	" <i>abc</i>	24 " <i>abbc</i>	
		14 " <i>AaBC</i>	78 " <i>AaBbCc</i>
		18 " <i>AaBc</i>	
		20 " <i>AabC</i>	
		16 " <i>Aabc</i>	

The whole expression contains 27 terms. Of these 8 are constant in all characters, and each appears on the average 10 times; 12 are constant in two characters, and hybrid in the third; each appears on the average 19 times; 6 are constant in one character and hybrid in the other two; each appears on the average 43 times. One form appears 78 times and is hybrid in all of the characters. The ratios 10, 19,

¹⁶ [Note that Mendel does not state the cotyledon-colour of the first crosses in this case; for as the coats were thick, it could not have been seen without opening or peeling the seeds.]

43, 78 agree so closely with the ratios 10, 20, 40, 80, or 1, 2, 4, 8, that this last undoubtedly represents the true value.

The development of the hybrids when the original parents differ in three characters results therefore according to the following expression:

$$\begin{aligned}&ABC + ABc + AbC + Abc + aBC \\&+ aBc + abC + abc + 2 ABCc \\&+ 2 AbCc + 2 aBCC + 2 abCc \\&+ 2 ABBc + 2 ABbc + 2 aBbC \\&+ 2 aBbc + 2 AaBC + 2 AaBc \\&+ 2 AabC + 2 Aabc + 4 ABbCc \\&+ 4 aBbCc + 4 AaBCC + 4 AabCc \\&+ 4 AaBbCc + 4 AaBbc + 8 AaBbCc.\end{aligned}$$

Here also is involved a combination series in which the expressions for the characters *A* and *a*, *B* and *b*, *C* and *c*, are united. The expressions

$$\begin{aligned}A + 2Aa + a \\B + 2Bb + b \\C + 2Cc + c\end{aligned}$$

give all the classes of the series. The constant combinations which occur therein agree with all combinations which are possible between the characters *A*, *B*, *C*, *a*, *b*, *c*; two thereof, *ABC* and *abc*, resemble the two original parental stocks.

In addition, further experiments were made with a smaller number of experimental plants in which the remaining characters by twos and threes were united as hybrids: all yielded approximately the same results. There is therefore no doubt that for the whole of the characters involved in the experiments the principle applies that the offspring of the hybrids in which several essentially different characters are combined exhibit the terms of a series of combinations, in which the developmental series for each pair of differentiating characters are united. It is demonstrated at the same time that the

relation of each pair of different characters in hybrid union is independent of the other differences in the two original parental stocks.

If n represents the number of the differentiating characters in the two original stocks, 3^n gives the number of terms of the combination series, 4^n the number of individuals which belong to the series, and 2^n the number of unions which remain constant. The series therefore contains, if the original stocks differ in four characters, $3^4 = 81$ classes, $4^4 = 256$ individuals, and $2^4 = 16$ constant forms; or, which is the same, among each 256 offspring of the hybrids there are 81 different combinations, 16 of which are constant.

All constant combinations which in Peas are possible by the combination of the said seven differentiating characters were actually obtained by repeated crossing. Their number is given by $2^7 = 128$. Thereby is simultaneously given the practical proof that the constant characters which appear in the several varieties of a group of plants may be obtained in all the associations which are possible according to the [mathematical] laws of combination, by means of repeated artificial fertilisation.

As regards the flowering time of the hybrids, the experiments are not yet concluded. It can, however, already be stated that the time stands almost exactly between those of the seed and pollen parents, and that the constitution of the hybrids with respect to this character probably follows the rule ascertained in the case of the other characters. The forms which are selected for experiments of this class must have a difference of at least twenty days from the middle flowering period of one to that of the other; furthermore, the seeds when sown must all be placed at the same depth in the earth, so that they may germinate

simultaneously. Also, during the whole flowering period, the more important variations in temperature must be taken into account, and the partial hastening or delaying of the flowering which may result therefrom. It is clear that this experiment presents many difficulties to be overcome and necessitates great attention.

If we endeavour to collate in a brief form the results arrived at, we find that those differentiating characters, which admit of easy and certain recognition in the experimental plants, all behave exactly alike in their hybrid associations. The offspring of the hybrids of each pair of differentiating characters are, one-half, hybrid again, while the other half are constant in equal proportions having the characters of the seed and pollen parents respectively. If several differentiating characters are combined by cross-fertilisation in a hybrid, the resulting offspring form the terms of a combination series in which the combination series for each pair of differentiating characters are united.

The uniformity of behaviour shown by the whole of the characters submitted to experiment permits, and fully justifies, the acceptance of the principle that a similar relation exists in the other characters which appear less sharply defined in plants, and therefore could not be included in the separate experiments. An experiment with peduncles of different lengths gave on the whole a fairly satisfactory result, although the differentiation and serial arrangement of the forms could not be effected with that certainty which is indispensable for correct experiment.

THE REPRODUCTIVE CELLS OF THE HYBRIDS

The results of the previously described experiments led to further ex-

periments, the results of which appear fitted to afford some conclusions as regards the composition of the egg and pollen cells of hybrids. An important clue is afforded in *Pisum* by the circumstance that among the progeny of the hybrids constant forms appear, and that this occurs, too, in respect of all combinations of the associated characters. So far as experience goes, we find it in every case confirmed that constant progeny can only be formed when the egg cells and the fertilising pollen are of like character, so that both are provided with the material for creating quite similar individuals, as is the case with the normal fertilisation of pure species. We must therefore regard it as certain that exactly similar factors must be at work also in the production of the constant forms in the hybrid plants. Since the various constant forms are produced in one plant, or even in one flower of a plant, the conclusion appears logical that in the ovaries of the hybrids there are formed as many sorts of egg cells, and in the anthers as many sorts of pollen cells, as there are possible constant combination forms, and that these egg and pollen cells agree in their internal composition with those of the separate forms.

In point of fact it is possible to demonstrate theoretically that this hypothesis would fully suffice to account for the development of the hybrids in the separate generations, if we might at the same time assume that the various kinds of egg and pollen cells were formed in the hybrids on the average in equal numbers.¹⁷

In order to bring these assumptions to an experimental proof, the following experiments were designed. Two forms which were constantly different

¹⁷ [This and the preceding paragraph contain the essence of the Mendelian principles of heredity.]

in the form of the seed and the colour of the albumen were united by fertilisation.

If the differentiating characters are again indicated as *A*, *B*, *a*, *b*, we have:

- AB*, seed parent;
- A*, form round;
- B*, albumen yellow.
- ab*, pollen parent;
- a*, form wrinkled;
- b*, albumen green.

The artificially fertilised seeds were sown together with several seeds of both original stocks, and the most vigorous examples were chosen for the reciprocal crossing. There were fertilised:

1. The hybrids with the pollen of *AB*.
2. The hybrids with the pollen of *ab*.
3. *AB* with the pollen of the hybrids.
4. *ab* with the pollen of the hybrids.

For each of these four experiments the whole of the flowers on three plants were fertilised. If the above theory be correct, there must be developed on the hybrids egg and pollen cells of the forms *AB*, *Ab*, *aB*, *ab*, and there would be combined:

1. The egg cells *AB*, *Ab*, *aB*, *ab* with the pollen cells *AB*.
2. The egg cells *AB*, *Ab*, *aB*, *ab* with the pollen cells *ab*.
3. The egg cells *AB* with the pollen cells *AB*, *Ab*, *aB*, *ab*.
4. The egg cells *ab* with the pollen cells *AB*, *Ab*, *aB*, *ab*.

From each of these experiments there could then result only the following forms:

1. $AB, ABb, AaB, AaBb.$
2. $AaBb, Aab, aBb, ab.$
3. $AB, ABB, AaB, AaBb.$
4. $AaBb, Aab, aBb, ab.$

If, furthermore, the several forms of the egg and pollen cells of the hybrids were produced on an average in equal numbers, then in each experiment the said four combinations should stand in the same ratio to each other. A perfect agreement in the numerical relations was, however, not to be expected, since in each fertilisation, even in normal cases, some egg cells remain undeveloped or subsequently die, and many even of the well-formed seeds fail to germinate when sown. The above assumption is also limited in so far that, while it demands the formation of an equal number of the various sorts of egg and pollen cells, it does not require that this should apply to each separate hybrid with mathematical exactness.

The first and second experiments had primarily the object of proving the composition of the hybrid egg cells, while the third and fourth experiments were to decide that of the pollen cells.¹⁸ As is shown by the above demonstration the first and third experiments and the second and fourth experiments should produce precisely the same combinations, and even in the second year the result should be partially visible in the form and colour of the artificially fertilised seed. In the first and third experiments the dominant characters of form and colour, A and B , appear in each union, and are also partly constant and partly in hybrid union with the recessive characters a and b , for which reason they must impress their peculiarity upon the whole of the seeds. All seeds should therefore appear round and yellow, if

the theory be justified. In the second and fourth experiments, on the other hand, one union is hybrid in form and in colour, and consequently the seeds are round and yellow; another is hybrid in form, but constant in the recessive character of colour, whence the seeds are round and green; the third is constant in the recessive character of form but hybrid in colour, consequently the seeds are wrinkled and yellow; the fourth is constant in both recessive characters, so that the seeds are wrinkled and green. In both these experiments there were consequently four sorts of seed to be expected—viz. round and yellow, round and green, wrinkled and yellow, wrinkled and green.

The crop fulfilled these expectations perfectly. There were obtained in the

1st Experiment, 98 exclusively round yellow seeds;

3rd Experiment, 94 exclusively round yellow seeds.

In the 2d Experiment, 31 round and yellow, 26 round and green, 27 wrinkled and yellow, 26 wrinkled and green seeds.

In the 4th Experiment, 24 round and yellow, 25 round and green, 22 wrinkled and yellow, 26 wrinkled and green seeds.

There could scarcely be now any doubt of the success of the experiment; the next generation must afford the final proof. From the seed sown there resulted for the first experiment 90 plants, and for the third 87 plants which fruited: these yielded for the

1st Exp. 3rd Exp.

20	25 round yellow seeds .	AB
23	19 round yellow and green seeds	ABb
25	22 round and wrinkled yellow seeds	AaB
22	21 round and wrinkled green and yellow seeds	$AaBb$

¹⁸ [To prove, namely, that both were similarly differentiated, and not one or other only.]

In the second and fourth experiments the round and yellow seeds yielded plants with round and wrinkled yellow and green seeds, $AaBb$.

From the round green seeds, plants resulted with round and wrinkled green seeds, Aab .

The wrinkled yellow seeds gave plants with wrinkled yellow and green seeds, aBb .

From the wrinkled green seeds plants were raised which yielded again only wrinkled and green seeds, ab .

Although in these two experiments likewise some seeds did not germinate, the figures arrived at already in the previous year were not affected thereby, since each kind of seed gave plants which, as regards their seed, were like each other and different from the others. There resulted therefore from the

2d Exp. 4th Exp.

31	24	plants of the form $AaBb$
26	25	" " " " Aab
27	22	" " " " aBb
26	27	" " " " ab

In all the experiments, therefore, there appeared all the forms which the proposed theory demands, and they came in nearly equal numbers.

In a further experiment the characters of flower-colour and length of stem were experimented upon, and selection was so made that in the third year of the experiment each character ought to appear in half of all the plants if the above theory were correct. A , B , a , b serve again as indicating the various characters.

A , violet-red flowers a , white flowers
 B , axis long. b , axis short.

The form Ab was fertilised with ab , which produced the hybrid Aab . Furthermore, aB was also fertilised with

ab , whence the hybrid aBb . In the second year, for further fertilisation, the hybrid Aab was used as seed parent, and hybrid aBb as pollen parent.

Seed parent, Aab .

Possible egg cells, Ab , ab .

Pollen parent, aBb .

Pollen cells, aB , ab .

From the fertilisation between the possible egg and pollen cells four combinations should result, viz.,

$$AaBb + aBb + Aab + ab.$$

From this it is perceived that, according to the above theory, in the third year of the experiment out of all the plants

Half should have violet-red flowers (Aa), Classes 1, 3.

Half should have white flowers (a), Classes 2, 4.

Half should have a long axis (Bb), Classes 1, 2.

Half should have a short axis (b), Classes 3, 4.

From 45 fertilisations of the second year 187 seeds resulted, of which only 166 reached the flowering stage in the third year. Among these the separate classes appeared in the numbers following:

Class	Color of flower	Stem	times
1	violet-red	long	47 times
2	white	long	40 "
3	violet-red	short	38 "
4	white	short	41 "

There subsequently appeared

The violet-red flower-colour (Aa) in 85 plants.

The white flower-colour (a) in 81 plants.

The long stem (*Bb*) in 87 plants.

The short stem (*b*) in 79 plants.

The theory adduced is therefore satisfactorily confirmed in this experiment also.

For the characters of form of pod, colour of pod, and position of flowers, experiments were also made on a small scale, and results obtained in perfect agreement. All combinations which were possible through the union of the differentiating characters duly appeared and in nearly equal numbers.

Experimentally, therefore, the theory is confirmed that *the pea hybrids form egg and pollen cells which, in their constitution, represent in equal numbers all constant forms which result from the combination of the characters united in fertilisation.*

The difference of the forms among the progeny of the hybrids, as well as the respective ratios of the numbers in which they are observed, find a sufficient explanation in the principle above deduced. The simplest case is afforded by the developmental series of each pair of differentiating characters. This series is represented by the expression $A + 2Aa + a$, in which *A* and *a* signify the forms with constant differentiating characters, and *Aa* the hybrid form of both. It includes in three different classes four individuals. In the formation of these, pollen and egg cells of the form *A* and *a* take part on the average equally in the fertilisation; hence each form [occurs] twice, since four individuals are formed. These participate consequently in the fertilisation

The pollen cells $A + A + a + a$

The egg cells $A + A + a + a$.

It remains, therefore, purely a matter of chance which of the two sorts of pollen will become united with each

separate egg cell. According, however, to the law of probability, it will always happen, on the average of many cases, that each pollen form, *A* and *a*, will unite equally often with each egg cell form, *A* and *a*, consequently one of the two pollen cells *A* in the fertilisation will meet with the egg cell *A* and the other with an egg cell *a*, and so likewise one pollen cell *a* will unite with an egg cell *A*, and the other with egg cell *a*.

$$\begin{array}{ccccc} \text{Pollen cells} & A & A & a & a \\ & \downarrow & \times & & \downarrow \\ \text{Egg cells} & A & A & a & a \end{array}$$

The result of the fertilisation may be made clear by putting the signs of the conjoined egg and pollen cells in the form of fractions, those for the pollen cells above and those for the egg cells below the line. We then have

$$\frac{A}{A} + \frac{A}{a} + \frac{a}{A} + \frac{a}{a}.$$

In the first and fourth term the egg and pollen cells are of like kind, consequently the product of their union must be constant, viz. *A* and *a*; in the second and third, on the other hand, there again results a union of the two differentiating characters of the stocks, consequently the forms resulting from these fertilisations are identical with those of the hybrid from which they sprang. *There occurs accordingly a repeated hybridisation.* This explains the striking fact that the hybrids are able to produce, besides the two parental forms, offspring which are like

themselves; $\frac{A}{a}$ and $\frac{a}{A}$ both give the

same union *Aa*, since, as already remarked above, it makes no difference in the result of fertilisation to which of the two characters the pollen or egg cells belong. We may write then

$$\frac{A}{A} + \frac{A}{a} + \frac{a}{A} + \frac{a}{a} = A + 2Aa + a.$$

This represents the average result of the self-fertilisation of the hybrids when two differentiating characters are united in them. In individual flowers and in individual plants, however, the ratios in which the forms of the series are produced may suffer not inconsiderable fluctuations.¹⁹ Apart from the fact that the numbers in which both sorts of egg cells occur in the seed vessels can only be regarded as equal on the average, it remains purely a matter of chance which of the two sorts of pollen may fertilise each separate egg cell. For this reason the separate values must necessarily be subject to fluctuations, and there are even extreme cases possible, as were described earlier in connection with the experiments on the form of the seed and the colour of the albumen. The true ratios of the numbers can only be ascertained by an average deduced from the sum of as many single values as possible; the greater the number, the more are merely chance effects eliminated.

The developmental series for hybrids in which two kinds of differentiating characters are united contains, among sixteen individuals, nine different forms, viz.,

$$AB + Ab + aB + ab + 2ABb + 2aBb + 2AaB + 2Aab + 4AaBb.$$

Between the differentiating characters of the original stocks, Aa and Bb , four constant combinations are possible, and consequently the hybrids produce the corresponding four forms of egg and pollen cells AB , Ab , aB , ab , and each

of these will on the average figure four times in the fertilisation, since sixteen individuals are included in the series. Therefore the participants in the fertilisation are

$$\begin{aligned} \text{Pollen cells } & AB + AB + AB + AB \\ & + Ab + Ab + Ab + Ab \\ & + aB + aB + aB + aB \\ & + ab + ab + ab + ab. \end{aligned}$$

$$\begin{aligned} \text{Egg cells } & AB + AB + AB + AB \\ & + Ab + Ab + Ab + Ab \\ & + aB + aB + aB + aB \\ & + ab + ab + ab + ab. \end{aligned}$$

In the process of fertilisation each pollen form unites on an average equally often with each egg cell form, so that each of the four pollen cells AB unites once with one of the forms of egg cell AB , Ab , aB , ab . In precisely the same way the rest of the pollen cells of the forms Ab , aB , ab unite with all the other egg cells. We obtain therefore

$$\begin{aligned} \frac{AB}{AB} & + \frac{AB}{Ab} + \frac{AB}{aB} + \frac{AB}{ab} + \frac{Ab}{AB} \\ & + \frac{Ab}{Ab} + \frac{Ab}{aB} + \frac{Ab}{ab} + \frac{aB}{AB} \\ & + \frac{aB}{Ab} + \frac{aB}{aB} + \frac{aB}{ab} + \frac{ab}{AB} \\ & + \frac{ab}{Ab} + \frac{ab}{aB} + \frac{ab}{ab}, \end{aligned}$$

or

$$\begin{aligned} AB & + ABb + AaB + AaBb + ABb \\ & + Ab + AaBb + Aab + AaB \\ & + AaBb + aB + aBb + AaBb \\ & + Aab + aBb + ab = AB + Ab \\ & + aB + ab + 2ABb + 2aBb \\ & + 2AaB + 2Aab + 4AaBb. \end{aligned}$$
²⁰

In precisely similar fashion is the developmental series of hybrids exhibited when three kinds of differentiating

¹⁹ [Whether segregation by such units is more than purely fortuitous may perhaps be determined by seriation.]

²⁰ [In the original the sign of equality (=) is here represented by +, evidently a misprint.]

characters are conjoined in them. The hybrids form eight various kinds of egg and pollen cells—*ABC*, *ABc*, *AbC*, *Abc*, *aBC*, *aBc*, *abC*, *abc*—and each pollen form unites itself again on the average once with each form of egg cell.

The law of combination of different characters, which governs the develop-

ment of the hybrids, finds therefore its foundation and explanation in the principle enunciated, that the hybrids produce egg cells and pollen cells which in equal numbers represent all constant forms which result from the combinations of the characters brought together in fertilisation.



Heredity in Populations and Pure Lines

A Contribution to the Solution of the Outstanding Questions in Selection

W. JOHANNSEN

Translated from *Ueber Erblichkeit in Populationen und in reinen Linien*, published by Gustav Fischer, Jena, 1903.

I have translated here only the final summary and discussion from Johannsen's long paper on pure lines, which was written in German. This thorough and meticulous investigation of the true significance of selection was a bombshell to evolutionary thought. The efficacy of selection in the production of new species had been one of the mainstays of Darwin's theory of evolution. Johannsen's studies demonstrated conclusively that selection could not extend the limits of previously established variability. This fact became important in arguments against Darwinism, and led to a period when selection was discredited as evolutionarily significant. The mutation theory became the new basis for explanation of evolutionary phenomena.

As has often happened in biology, the final solution of the problem involved a reconciliation of the two viewpoints. Mutation (as a source of variants) and selection (as a method of elimination of some but not all variants) provide the modern basis for explanation of the process of evolution. We owe to Johannsen our modern viewpoint of selection as a primarily passive process, which eliminates but does not produce variations.

Although Johannsen uses the German word "Typus" throughout his paper with reference to his pure lines, I have substituted his own term, "genotype," invented at a later date. To Johannsen goes the credit as well for inventing the word "gene." It should be noted that he wrestles with the various names that had been proposed for the hereditary particles in this paper, on p. 26, but does not at this time suggest the term gene.

SUMMARY AND CONCLUSIONS

ALL THAT WILL BE DISCUSSED HERE gives at one and the same time a complete confirmation and a total elucidation of Galton's well-known law of regression, which concerns the relationship between parent and offspring. Other regression relationships do not concern us here.

Insofar as my research material is concerned, it agrees very well with Galton's Law. This law states that individuals differing from the average character of the population produce offspring which, on the average, differ to a lesser degree but in the same direction from the average as their parents. Selection within a population causes a greater or lesser shift in the direction of the selection of the average for the characteristics around which the individuals concerned are fluctuating.

While as a consequence of this I am not able to continue to regard the population as completely uniform, nevertheless my material can be broken up into "pure lines." It has been demonstrated that in all cases within the pure lines the retrogression mentioned above has been completed: Selection within the pure lines has produced no new shift in genotype.

The shift in the average for a characteristic, which selection in a population can usually produce, is thus an indication that the total population—at least in my material—consists of different "lines" whose genotypes can be more or less differentiated. In the course of ordinary selection a population would become impure; this result is a consequence of the incomplete isolation of these lines, whose genotypes cause deviation of the average character of the population in their directions.

The typical, well-known results of selection, that is, step-wise progress in

the direction of the selection in the course of each generation, therefore depends upon step-wise progression in each generation of the differing lines concerned. It is now easily understood that the action of selection cannot go beyond the known limits—it must stop when the purification, or, practically speaking, the isolation of the most strongly divergent pure line is complete. In this connection it must be pointed out that one can never ascertain with certainty the existence of only a single genotype in a sample solely on the basis of concordance between the table or curve of variation shown by that sample and the numerical proportions of the binomial formula. The variation curve of individuals representing a racially pure population in the ordinary sense, frequently, indeed perhaps in most instances, can be shown to be the result of numerous genotypes representing the various pure lines of the population. The average value thus does not always have the significance of a true genotype. A great deficiency of a purely statistical approach is obvious in this regard.

For this reason, I have attempted throughout this paper to distinguish sharply between the concept of the mean (average character, average values, and so on) and the concept of the genotype. The confusion of these two thoroughly different concepts has only too frequently caused misunderstanding and erroneous inferences, perhaps not only in the field of heredity. It must be conceded that it can often be extremely difficult to distinguish between these two concepts without detailed analysis; and in pure lines the two concepts frequently cover the same area. The numerical expression of a genotype is frequently, but by no means always, an average value.

In the case of morphological characters—at least with the entire series of those whose value in systematic investigations has been generally recognized—the distinction between the different genotypes is such that the single individual can usually be recognized, in spite of its variations, as belonging to one or another of the most narrow systematic categories (for example, the “subspecies” of Jordan).

These morphological types can usually be organized into precise variation series only with great difficulty, for a mixture of individuals of different genotypes might be combined with a series of individuals which belong to a unique genotype. A mixture such as the *Oenothera* forms of de Vries or Raunkiaer's *Taraxacum* “Geschlechter”¹ gives with regard to the essential morphological characteristics a different picture than a pure culture of a single form.

With regard to all sorts of characters of a more physiological sort—the non-botanical characters of Hj. Nilssons—such as, for example, most height and other proportions, biochemical properties, reliable numerical relationships, and others, we have a different situation. The distinct, actually existant genotypes, easily demonstrated through isolated cultivation, show only quantitative differences, so that the variation curves of the different genotypes overlap, and one has the transgressive curves of Hugo de Vries. A mixture of individuals, which belong to genotypes clearly distinct with respect to one of these characteristics (compare small and large, as well as narrow and broad beans) can very easily form so continuous a variation series that it is not possible to recognize

directly the distinctions between genotypes, and the average value will be erroneously regarded as that of a single genotype. In cases such as these it is impossible to distinguish the genotype to which a single individual belongs. Table 1 is a good illustration of this point.

It is for these reasons, which have been more or less clearly recognized or just sensed, that the study of the characters I might call “truly” morphological, described above, have provided the center of gravity for systematics. The more physiological characters have entered into the sphere of interest of the systematist only in recent years, particularly with regard to lower forms. These reasons also explain why the students of mutation have found the mainstay of their researches in true morphological characters. On the other hand, these characteristics, which essentially determine the entire habitus of the plant, either cannot or can only partially be expressed in numerical measures, and this almost invariably reduces their value to within the limits of the exact methods of measurements and calculation.

Biometrical research, that is, the investigation of the laws of variation and heredity, has thus included primarily the more physiological characters or in general the characters Bateson called “meristic,” that is, those which can be expressed clearly in numbers, such as size or weight. And here, where comparisons of individual with individual does not enable one to distinguish differences in genotype from the manifestations of a fluctuating variability, is the stronghold of the Galton-Pearson concept: Here one must—if one fails to consider pure lines—necessarily come to the conclusion that selection of strongly variant individuals (either plus or minus variants)

¹ Raunkiaer, C., “Kimdannelsc uden Befrugtning hos Mælkebotte” (*Botan. Tidskrift*, Bd. XXV, Kopenhagen, 1903), pp. 109–119.

TABLE I

Variation in Offspring, Divided into Classes
by Centigrams

Weight Groups of Parent Seeds (1901) in Milligrams	Standard Deviation										Totals	\pm							
	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90		
150-250			1	3	12	29	61	38	25	11	71	20	2			180	69.6		
250-350		2	13	37	58	133	189	195	115	213	69	20	4			835	87.0		
350-450	5	6	11	36	139	278	498	584	372	234	120	76	34	17	3	2238	85.1		
450-550		4	20	37	101	204	287	204	103	127	102	66	34	12	6	1138	91.8		
550-650		1	9	14	51	79	16	37	71	104	105	75	45	19	12	5	609	102.5	
650-750		2	3	16	2	3	16	16	107	263	608	1068	1278	977	622	306	135	2	494
Total Material	5	8	30	107	263	608	1068	1278	977	622	306	135	52	24	9	2	5494	95.3	

TABLE I. The relationship of the weight of offspring to the weight of the parent seed. The seeds produced in 1901 were divided into weight classes and then planted. The seeds produced by the members of each weight class were weighed and classified, with the results shown under "Variation in Offspring."

can effect an actual change in the genotype under consideration. It is obvious that this concept, which has been completely acceptable up to this time and which the biometricalians Weldon and Pearson have supported, must hinder the acceptance of mutations as something other than and as important as fluctuating variation. With all of the accumulated statistical knowledge of heredity in populations the acceptance of the mutation theory was perhaps felt to be not necessary for biology. I say "perhaps" here to meet to a certain extent the objections of biometricalians. As for myself, the magnificent experiments of Hugo de Vries have proven the existence of mutations beyond the shadow of a doubt.

It appears to be obvious from the research results I have presented here that the basis of the Galton-Pearson Law, concerning the relationship between parents and offspring, is something other than that which has been taken for granted up to now. The individual peculiarities of the parents, grandparents, or any other ancestor, has—insofar as my researches are concerned—no influence on the average characteristics of the offspring. It is the genotype of the line working in intimate conjunction with the external environment of a specific locality at a particular time that determines the average characteristics of an individual. The "line" is accordingly "completely constant and highly variable," as de Vries has so clearly shown in a similar situation, although apparently in a paradoxical fashion.²

At the same time, it must not be implied that the pure line will be absolutely constant.

First there is the possibility that selection of fluctuating variants through very many generations can eventually shift the genotype of a line. This has

² Die Mutationstheorie, vol. 1, p. 97.

not been positively demonstrated—the statements of biometricalists apply, as has been frequently pointed out, to populations which are not but could be divided into pure lines. The burden of proof for this possibility lies upon those who would wish to verify the efficacy of this kind of selection.

Second we must consider cross-breeding—to take part in which the pure lines must forfeit their pure condition! The whole hybrid question is not, however, part of our discussion.

Third we come to mutations, the possibility of irregular changes in genotype. To define them would be premature in the greatest degree. Their existence in a greater diversity of organisms must first be substantiated. That they do so occur cannot be doubted, in my opinion; I hope to present specific, positive proof in a later publication. No more will be said here than that a mutation in a given direction cannot be specifically identified strictly on the basis of offspring of individuals which deviate irregularly in that direction.

At this point I must refer to the ticklish problem of what might explain the statement of de Vries that one frequently observes "minus" variations are predominant in newly discovered genotypes—an event that not without reason has aroused the skepticism of biometricalists. It is to be hoped that the studies presented here will shed some light on this problem, which perhaps only appears to destroy the boundary between fluctuating variation and mutation.

(Postscript: In the final part of his *Mutationstheorie* — which appeared during the editing of this work—de Vries (l.c., pp. 503–504) has shown in a most ingenious way how in most cases mutations are first expressed. Therein lies an outstandingly impor-

tant instance for the explanation of the relationship just discussed.)

Hugo de Vries has included in his *Mutationstheorie* (vol. 1, p. 368 ff.) a separate chapter concerning "Nourishment and Natural Selection" in which the consequences of a rich or scanty nourishment by the maternal plant is discussed. I have no doubt that actual or imaginary differences in nourishment occurring simultaneously with the presence or absence of selection would account for de Vries' example. Also, the phase of ontogeny which de Vries called the "sensitive period" is of particular interest. I could cite a similar phenomenon in my research material only with the greatest difficulty. It should be understood that it is not my intention to try to explain through the "principle of pure lines" and nothing else all the differences in characteristics which selection in conjunction with extreme or experimentally designed habitats might produce. In this regard, which is of strong interest to Neo-Lamarckians, there is still very much research to be done—and certainly with the use of genuinely pure lines as a research material.

My primary purpose is to shed some light on the Galtonian regression between ancestors and descendants, and I believe that my material, which evidently has its natural peculiarities analogous to those of Galton, has its value as a basis for analysis of the Galtonian laws applying to populations. My statements conflict neither with Galton's statements nor with those of de Vries.

If my investigations are sound, and their significance is grasped beyond the special case here discussed, the general results of this work would form a not unimportant support for the modern concepts of Bateson and de Vries on the great significance of "discontinuous" variations, or "mutations," for

the theory of evolution. For a selection in cases such as mine is effective only in so far as it selects out representatives of an already existant genotype. These genotypes would not be successively originated through the retention of those individuals which vary in the desired direction; they would merely be found and isolated.

The knowledge that has been gained from studies on pure lines, combined with a knowledge of hybridization, must serve as the starting point in the case of studies on heredity within population in which pure lines can not be completely isolated as a consequence of the necessity of constant cross-fertilization or hybridization. This knowledge is, as has been pointed out earlier, in complete agreement with the basic ideas in the great work of de Vries—as has been seen, my concepts have been arrived at via a somewhat different path than that followed by de Vries, and it is also important to note, based on a different kind of information.

In addition the important question of correlative variation takes on a somewhat changed character depending upon whether one works with pure lines or with populations. In the latter case a given “ratio of correlation” (Pearson’s term) will not necessarily represent a strongly legitimate relationship, as I have sought to demonstrate earlier. An indicated correlative relationship is much more significant within a pure line. My summation table speaks very well for this concept, in that it was not possible to change through selection within pure lines the correlation between length and width of the beans, while it was simple to isolate truly different genotypes, as for example narrow and broad forms, from the original population, which appeared to be entirely homogeneous.

Again, we have to reckon with the

possibility of mutation; for thereby even the strongest correlative relationship could be destroyed. I do not wish to take up this question at this time; in a later publication I hope to shed more light on it, using the principle of pure lines as the basis for the research.

I would be sorry indeed if the reader of this work would come to feel that the value of the significant work of Galton, Pearson, and other biometrical research workers were to be placed in doubt. I would not have the audacity to criticize the treatment which Pearson in particular has given to the question of the ancestral influence within a specific population. I do think, however, that the principle of pure lines in the hands of a man such as Pearson can carry biometric studies much farther along than his studies of populations. Obviously the relationships studied by Pearson have great scientific significance and they have considerable practical value as well—but they are not suitable to illuminate completely the fundamental laws of heredity.

And what particularly affects Galton’s research, in my estimation, is that the results presented here support in a beautiful way the basic ideas of Galton’s “Stirp” theory,³ which was already worked out in 1876. This law includes almost all that is of actual value in the more recent Weismann theory on the “Continuity of the Germplasm.” That the speculation of Weismann⁴ could overshadow the

³ Galton’s theory is known to me from his original paper in *Revue Scientifique*, vol. 10, 1876, p. 198 (“Theorie de l’hérédité”).

⁴ The position of biometricalians on “Weismannism” has been clearly evaluated by Pearson in his characterization of this movement in “Socialism and Natural Selection” (*Fortnightly Review*, July, 1894. Reprinted in Pearson’s *Chances of Death*, vol. 1, 1897, p. 104). I do not plan to go further into this question at this time.

more simply put but no less ingenious and quite original idea of Galton's is perhaps due to some extent to Galton himself, for he has not seen fit in his more recent publications to adhere to his Stirp theory in the light of research progress. The Stirp theory does not correlate too well with Galton's Law of Regression, it is true, but it could scarcely be better supported or illustrated than by the results which I have described: a usually complete regression to the genotype of a pure line seems to me the most beautiful evidence for a slightly modified Stirp concept. It is true that Galton's Stirp concept cannot be maintained unchanged. Although Weismann⁵ very recently regarded Galton as the "voice" of cellular limitation through "Determinants" — or however one might name these theoretical hereditary corpuscles, de Vries deserves the great credit for having recognized the unitary nature of hereditary particles, which he called "pangenes"—a concept he first published in 1889⁶ and further advanced in the "Mutationstheorie." It seems to me that the Galton-de Vries theory is the only truly useful theory of heredity.

⁵ Weismann, *Vorträge über die Deszendenztheorie*, 1902, p. 421.

⁶ de Vries, "Intracelluläre Pangenesis," Jena, 1889.

Should the present publication be successful in bringing the principle of pure lines recognition as an absolutely necessary principle in truly intensive research in the study of heredity, then its highest purpose would be achieved. Later publications will attempt to illuminate the activity of lines which vary polymodally. I have investigated only unimodal variation in this paper,⁷ in order to present my concept in its simplest instance.

The train of thought which underlies this investigation is expressed in its simplicity most clearly by the often cited words of Goethe:

"Dich im Unendlichen zu finden
Musst unterscheiden und dann
verbinden."⁸

Vilmorin has emphasized the differentiation of the parts, Galton has demonstrated the legitimate basis for recombination; I have tried here to combine the points of view for which these two ingenious investigators are honored.

⁷ de Vries has published a special case concerning heredity in a pure "bimodal" line in his *Mutationstheorie* (vol. 2, p. 509), based on a short communication of mine.

⁸ "Before the infinite can be thine
You must first break it 'down and then
re-combine."



The Chromosomes in Heredity

WALTER S. SUTTON

Reprinted by publisher's permission from *Biological Bulletin*, vol. 4, 1903, pp. 231-251.

It has often happened that two people have made practically identical discoveries and published their findings almost simultaneously. This has been particularly true in genetics, which finds its modern origin in the tripartite publicity Mendel's paper received at the turn of the century. The hypothesis advanced in this paper by Sutton was also conceived by Boveri and published in the same year. As a consequence, the theory that the hereditary particles are borne by the chromosomes is known as the "Sutton-Boveri Hypothesis."

Sutton does not provide any original data in this paper. It represents a different type of contribution from that seen in the first two papers in this collection, which are primarily analyses of research results. Sutton has performed a vital function here; one that is almost as vital as research itself in biological progress. When an author takes a series of apparently unrelated facts and ideas from two areas of investigation, combines them so that they make new sense, and develops a new hypothesis from the combination, he not only aids in the advance of both fields but also is quite likely to open up a new one. This is the kind of contribution Darwin made in his "Origin of Species," which is almost entirely a correlation and synthesis of several diverse groups of facts. In Sutton's paper you will see this development of relationships between the fields of cytology and heredity, which, at the time Sutton wrote, were considered to be fairly divergent from each other, in that no research techniques were shared. Today the two fields are permanently linked, many research methods have developed to handle mutual problems, and Sutton's paper can be considered the beginning of the field called cytogenetics.

You should note as you read the sequence and development of Sutton's arguments, and the clear logic and reasoning he employs. This paper is a good model to follow in the preparation of a study involving synthesis and correlation. It is comparatively easy to read and understand. Caution should be used in the last few pages, for Sutton gets involved in generalities that go well beyond the limits of his analysis. The value of this paper lies in the thoroughness with which Sutton demonstrates the validity of his hypothesis, and not in the attempts to explain many other problems through the use of the hypothesis.

IN A RECENT ANNOUNCEMENT OF some results of a critical study of the chromosomes in the various cell-generations of *Brachystola*¹ the author briefly called attention to a possible relation between the phenomena there described and certain conclusions first drawn from observations on plant hybrids by Gregor Mendel² in 1865, and recently confirmed by a number of able investigators. Further attention has already been called to the theoretical aspects of the subject in a brief communication by Professor E. B. Wilson.³ The present paper is devoted to a more detailed discussion of these aspects, the speculative character of which may be justified by the attempt to indicate certain lines of work calculated to test the validity of the conclusions drawn. The general conceptions here advanced were evolved purely from cytological data, before the author had knowledge of the Mendelian principles, and are now presented as the contribution of a cytologist who can make no pretensions to complete familiarity with the results of experimental studies on heredity. As will appear hereafter, they completely satisfy the conditions in typical Mendelian cases, and it seems that many of the known deviations from the Mendelian type may be explained by easily conceivable variations from the normal chromosomal processes.

It has long been admitted that we

¹ Sutton, Walter S., "On the Morphology of the Chromosome Group in *Brachystola magna*," *Biol. Bull.*, IV., 1, 1902.

² Mendel, Gregor Johann, "Versuche über Pflanzen-Hybriden," *Verb. naturf. Vers. in Brünn* IV., and in Osterwald's *Klassiker der exakten Wissenschaft*. English translation in *Journ. Roy. Hort. Soc.*, XXVI., 1901. Later reprinted with modifications and corrections in Bateson's "Mendel's Principles of Heredity," Cambridge, 1902, p. 40.

³ Wilson, E. B., "Mendel's Principles of Heredity and the Maturation of the Germ-Cells," *Science*, XVI., 416.

must look to the organization of the germ-cells for the ultimate determination of hereditary phenomena. Mendel fully appreciated this fact and even instituted special experiments to determine the nature of that organization. From them he drew the brilliant conclusion that, while, in the organism, maternal and paternal potentialities are present in the field of each character, *the germ-cells in respect to each character are pure*. Little was then known of the nature of cell-division, and Mendel attempted no comparisons in that direction; but to those who in recent years have revived and extended his results the probability of a relation between cell-organization and cell-division has repeatedly occurred. Bateson⁴ clearly states his impression in this regard in the following words: "It is impossible to be presented with the fact that in Mendelian cases the cross-bred produces on an average *equal* numbers of gametes of each kind, that is to say, a symmetrical result, without suspecting that this fact must correspond with some symmetrical figure of distribution of the gametes in the cell divisions by which they are produced."

Nearly a year ago it became apparent to the author that the high degree of organization in the chromosome-group of the germ-cells as shown in *Brachystola* could scarcely be without definite significance in inheritance, for, as shown in the paper⁵ already referred to, it had appeared that:

1. The chromosome group of the presynaptic germ-cells is made up of two equivalent chromosome-series, and that strong ground exists for the conclusion that one of these is paternal and the other maternal.

⁴ Bateson, W., "Mendel's Principles of Heredity," Cambridge, 1902, p. 30.

⁵ Sutton, W. S., *loc. cit.*

2. The process of synapsis (pseudo-reduction) consists in the union in pairs of the homologous members (*i. e.*, those that correspond in size) of the two series.⁶

3. The first post-synaptic or maturation mitosis is equational and hence results in no chromosomal differentiation.

4. The second post-synaptic division is a reducing division, resulting in the separation of the chromosomes which have conjugated in synapsis, and their relegation to different germ-cells.

5. The chromosomes retain a morphological individuality throughout the various cell-divisions.

It is well known that in the eggs of many forms the maternal and paternal chromosome groups remain distinctly independent of each other for a considerable number of cleavage-mitoses, and with this fact in mind the author was at first inclined to conclude that in the reducing divisions all the maternal chromosomes must pass to one pole and all the paternal ones to the other, and that the germ-cells are thus divided into two categories which might be described as maternal and paternal respectively. But this conception, which is identical with that recently brought forward by Cannon,⁷ was soon seen to be at variance with many well-known facts of breeding; thus:

1. If the germ-cells of hybrids are of pure descent, no amount of cross-

⁶ The conclusion that synapsis involves a union of paternal and maternal chromosomes in pairs was first reached by Montgomery in 1901.

Montgomery, T. H., Jr., "A Study of the Chromosomes of the Germ-Cells of Metazoa," *Trans. Amer. Phil. Soc.*, XX.

⁷ Cannon, W. A., "A Cytological Basis for the Mendelian Laws," *Bull. Torrey Botanical Club*, 29, 1902.

breeding could accomplish more than the condition of a first-cross.

2. If any animal or plant has but two categories of germ-cells, there can be only four different combinations in the offspring of a single pair.

3. If either maternal or paternal chromosomes are entirely excluded from every ripe germ-cell, an individual cannot receive chromosomes (qualities) from more than one ancestor in each generation of each of the parental lines of descent, *e. g.*, could not inherit chromosomes (qualities) from both paternal or both maternal grandparents.

Moved by these considerations a more careful study was made of the whole division-process, including the positions of the chromosomes in the nucleus before division, the origin and formation of the spindle, the relative positions of the chromosomes and the diverging centrosomes, and the point of attachment of the spindle fibers to the chromosomes. The results gave no evidence in favor of parental purity of the gametic chromatin as a whole. On the contrary, many points were discovered which strongly indicate⁸ that the position of the bivalent chromosomes in the equatorial plate of the

⁸ Absolute proof is impossible in a pure-bred form on account of the impossibility of distinguishing between maternal and paternal members of any synaptic pair. If, however, such hybrids as those obtained by Moenkhaus (Moenkhaus, W. J., "Early Development in Certain Hybrid Species," Report of Second Meeting of Naturalists at Chicago, *Science*, XIII., 323), with fishes can be reared to sexual maturity absolute proof of this point may be expected. This observer was able in the early cells of certain fish hybrids to distinguish the maternal from the paternal chromosomes by differences in form, and if the same can be done in the maturation-divisions the question of the distribution of chromosomes in reduction becomes a very simple matter of observation.

reducing division is purely a matter of chance—that is, that any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the positions of other pairs—and hence that a large number of different combinations of maternal and paternal chromosomes are possible in the mature germ-products of an individual. To illustrate this, we may consider a form having eight chromosomes in the somatic and presynaptic germ-cells and consequently four in the ripe germ-products. The germ-cell series of the species in general may be designated by the letters A, B, C, D, and any cleavage nucleus may be considered as containing chromosomes *A*, *B*, *C*, *D* from the father and *a*, *b*, *c*, *d*, from the mother. Synapsis being the union of homologues would result in the formation of the bivalent chromosomes *Aa*, *Bb*, *Cc*, *Dd*, which would again be resolved into their components by the reducing division. Each of the ripe germ-cells arising from the reduction divisions must receive one member from each

of the synaptic pairs, but there are sixteen possible combinations of maternal and paternal chromosomes that will form a complete series, to wit: *a*, *B*, *C*, *D*; and their conjugates *A*, *b*, *c*, *d*; *A*, *B*, *C*, *D*. Hence instead of two kinds of gametes an organism with four chromosomes in its reduced series may give rise to 16 different kinds; and the offspring of two unrelated individuals may present 16×16 or 256 combinations, instead of the four to which it would be limited by a hypothesis of parental purity of gametes. Few organisms, moreover, have so few as 8 chromosomes, and since each additional pair doubles the number of possible combinations in the germ-products⁹ and quadruples that of the zygotes it is

⁹ The number of possible combinations in the germ-products of a single individual of any species is represented by the simple formula 2^n in which n represents the number of chromosomes in the reduced series.

TABLE 1

Chromosomes		Combinations in Gametes	Combinations in Zygotes
Somatic Series	Reduced Series		
2	1	2	4
4	2	4	16
6	3	8	64
8	4	16	256
10	5	32	1,024
12	6	64	4,096
14	7	128	16,384
16	8	256	65,536
18	9	512	262,144
20	10	1,024	1,048,576
22	11	2,048	4,194,304
24	12	4,096	16,777,216
26	13	8,192	67,108,864
28	14	16,384	268,435,456
30	15	32,768	1,073,741,824
32	16	65,536	4,294,967,296
34	17	131,072	17,179,869,184
36	18	262,144	68,719,476,736

plain that in the ordinary form having from 24 to 36 chromosomes, the possibilities are immense. Table I below shows the number of possible combinations in forms having from 2 to 36 chromosomes in the presynaptic cells.

Thus if Bardeleben's estimate of sixteen chromosomes for man (the lowest estimate that has been made) be correct, each individual is capable of producing 256 different kinds of germ-products with reference to their chromosome combinations, and the numbers of combinations possible in the offspring of a single pair is 256×256 or 65,536; while *Toxopneustes*, with 36 chromosomes, has a possibility of 262,144 and 68,719,476,736 different combinations in the gametes of a single individual and the zygotes of a pair respectively. It is this possibility of so great a number of combinations of maternal and paternal chromosomes in the gametes which serves to bring the chromosome-theory into final relation with the known facts of heredity; for Mendel himself followed out the actual combinations of two and three distinctive characters and found them to be inherited independently of one another and to present a great variety of combinations in the second generation.

The constant size-differences observed in the chromosomes of *Brachystola* early led me to the suspicion, which, however, a study of spermatogenesis alone could not confirm, that the individual chromosomes of the reduced series play different rôles in development. The confirmation of this surmise appeared later in the results obtained by Boveri¹⁰ in a study of

larvæ actually lacking in certain chromosomes of the normal series, which seem to leave no alternative to the conclusion that the chromosomes differ qualitatively and as individuals represent distinct potentialities. Accepting this conclusion we should be able to find an exact correspondence between the behavior in inheritance of any chromosome and that of the characters associated with it in the organism.

In regard to the characters, Mendel found that, if a hybrid produced by crossing two individuals differing in a particular character be self-fertilized, the offspring, in most cases, conform to a perfectly definite rule as regards the differential character. Representing the character as seen in one of the original parents by the letter A and that of the other by a, then all the offspring arising by self-fertilization of the hybrid are represented from the standpoint of the given character by the formula AA : 2Aa : aa.—that is, one fourth receive only the character of one of the original pure-bred parents, one fourth only that of the other; while one half the number receive the characters of both original parents and hence present the condition of the hybrid from which they sprang.

We have not heretofore possessed graphic formulæ to express the combinations of chromosomes in similar breeding experiments, but it is clear from the data already given that such formulæ may now be constructed. The reduced chromosome series in *Brachystola* is made up of eleven members, no two of which are exactly of the same size. These I distinguished in my previous paper by the letters A, B, C, . . . K. In the unreduced series there are

¹⁰ Boveri, Th., "Ueber Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns," *Verb. d. Phys.-Med. Ges. zu Würzburg*, N. F., Bd. XXXV., 1902. It appears from a personal letter that Boveri had noted the correspondence between chromosomal behavior as de-

ducible from his experiments and the results on plant hybrids—as indicated also in footnote 1, *l. c.*, p. 81.

twenty-two elements¹¹ which can be seen to make up two series like that of the mature germ-cells, and hence may be designated as A, B, C . . . K + A, B, C . . . K. Synapsis results in the union of homologues and the production of a single series of double-elements thus: AA, BB, CC . . . KK, and the reducing division affects the separation of these pairs so that one member of each passes to each of the resulting germ-products.

There is reason to believe that the division-products of a given chromosome in *Brachystola* maintain in their respective series the same size relation as did the parent element; and this, taken together with the evidence that the various chromosomes of the series represent distinctive potentialities, make it probable that a given size-relation is characteristic of the physical basis of a definite set of characters. But each chromosome of any reduced series in the species has a homologue in any other series, and from the above consideration it should follow that these homologues cover the same field in development. If this be the case chromosome *A* from the father and its homologue, chromosome *a*, from the mother in the presynaptic cells of the offspring may be regarded as the physical bases of the antagonistic unit-characters *A* and *a* of father and mother respectively. In synapsis, copulation of the homologues gives rise to the bivalent chromosome *Aa*, which as is indicated above would, in the reducing division, be separated into the components *A* and *a*. These would in all cases pass to different germ-products and hence in a monoecious form we should have four sorts of gametes,

<i>A</i> ♂	<i>a</i> ♂
<i>A</i> ♀	<i>a</i> ♀

which would yield four combinations,

$$\begin{aligned} A \delta + A \varphi &= AA \\ A \delta + a \varphi &= Aa \\ a \delta + A \varphi &= aA \\ a \delta + a \varphi &= aa \end{aligned}$$

Since the second and third of these are alike the result would be expressed by the formula *AA* : *2Aa* : *aa* which is the same as that given for any character in a Mendelian case. *Thus the phenomena of germ-cell division and of heredity are seen to have the same essential features, viz., purity of units (chromosomes, characters) and the independent transmission of the same;* while as a corollary, it follows in each case that each of the two antagonistic units (chromosomes, characters) is contained by exactly half the gametes produced.

The observations which deal with characters have been made chiefly upon hybrids, while the cytological data are the result of study of a pure-bred form; but the correlation of the two is justified by the observation of Cannon¹² that the maturation mitoses of fertile hybrids are normal. This being the case it is necessary to conclude, as Cannon has already pointed out, that the course of variations in hybrids either is a result of normal maturation processes or is entirely independent of the nature of those divisions. If we conclude from the evidence already given that the double basis of hybrid characters is to be found in the pairs of homologous chromosomes of the presynaptic germ-cells, then we must also conclude that in pure-bred forms likewise, the paired arrangement of the chromosomes indicates a dual basis for each character. In a hypothetical species breeding absolutely true, therefore, all the chromosomes or subdivisions of chromo-

¹¹ Disregarding the accessory chromosome which takes no part in synapsis.

¹² Cannon, W. A., *loc. cit.*

somes representing any given character would have to be exactly alike, since the combination of any two of them would produce a uniform result. As a matter of fact, however, specific characters are not found to be constant quantities but vary within certain limits; and many of the variations are known to be inheritable. Hence it seems highly probable that homologous chromatin-entities are not usually of strictly uniform constitution, but present minor variations corresponding to the various expressions of the character they represent. In other words, it is probable that specific differences and individual variations are alike traceable to a common source, which is a difference in the constitution of homologous chromatin-entities. Slight differences in homologues would mean corresponding, slight variations in the character concerned—a correspondence which is actually seen in cases of inbreeding, where variation is well known to be minimized and where obviously in the case of many of the chromosome pairs both members must be derived from the same chromosome of a recent common ancestor and hence be practically identical.

In the various forms of parthenogenesis we meet the closest kind of inbreeding and a brief consideration of the variability to be expected in each, from the standpoint of the chromosome theory, may serve as a guide to such research as will test the validity of the latter. The simplest form, of which chemical parthenogenesis in sea-urchins is an example, is that in which the organism has only a single chromosome series, to be represented by $A, B, C, D \dots N$. Thus far no recognized cases of this type have been reared to sexual maturity, but it is to be expected that no reducing division will be found in the maturation of such

forms, and that their parthenogenetic offspring will exactly resemble the immediate parent.

In cases of natural parthenogenesis which are accompanied by the reentrance of the second polar body and its fusion with the egg-nucleus (or its failure to form) there must be a double chromosome series; but we may distinguish two classes according as the reducing process is accomplished in the first or the second maturation division.¹³ If reduction is accomplished in the first division, one half the chromosomes of the oögonia are thrown out and lost in the first polar body. The second division, being equational, would result in a polar body which would be the exact duplicate of the egg-nucleus as far as chromosomes are concerned and which accordingly, by its reentrance would add nothing new to the egg-series. The series after fusion would, therefore, be represented by the letters $A, B, C, D \dots N + A, B, C, D \dots N$. If such a type of parthenogenesis were to follow sexual reproduction, the first generation of offspring might be expected to differ materially from the parent by reason of the casting out, in the first polar body, of chromosomes representing certain dominant characters, and the consequent appearance in the offspring of the corresponding recessives. Subsequent parthenogenetic generations, however, would in each case be endowed with a chromosome series exactly similar to that of the immediate parent and accordingly might be expected to show the same characters.

In case the second division of a par-

¹³ Either must be regarded as possible in cases where we have no definite knowledge since it is regularly described as the second in the Orthoptera (McClung, Sutton) and Copepoda (Rückert, Häcker) while in the Hemiptera-Heteroptera it is believed to be the first (Paulmier, Montgomery).

thenogenetic egg were the reducing division, the reentrance or suppression of the second polar body would accomplish the restoration of the oögonial chromosome-series. In this case the first parthenogenetic generation might be expected to duplicate the characters of the parent (if environmental conditions remained unchanged) and little or no variability would be expected as long as parthenogenesis persisted.

In relation to these problems there is great need of a simultaneous study of the germ-cell divisions and the variation of periodically parthenogenetic forms.

We have seen reason, in the foregoing considerations, to believe that there is a definite relation between chromosomes and allelomorphs¹⁴ or unit characters but we have not before inquired whether an entire chromosome or only a part of one is to be regarded as the basis of a single allelomorph. The answer must unquestionably be in favor of the latter possibility, for otherwise the number of distinct characters possessed by an individual could not exceed the number of chromosomes in the germ-products; which is undoubtedly contrary to fact. We must, therefore, assume that some chromosomes at least are related to a number of different allelomorphs. If then, the chromosomes permanently retain their individuality, it follows that all the allelomorphs represented by any one chromosome must be inherited together. On the other hand, it is not necessary to assume that all must be apparent in the organism, for here the question of dominance enters and it is not yet known that dominance is a function of an entire chromosome. It is conceivable that the chromosome may be divisible into smaller entities (some-

what as Weismann assumes), which represent the allelomorphs and may be dominant or recessive independently. In this way the same chromosome might at one time represent both dominant and recessive allelomorphs.

Such a conception infinitely increases the number of possible combinations of characters *as actually seen* in the individuals and unfortunately at the same time increases the difficulty of determining what characters are inherited together, since usually recessive chromatin entities (allelomorphs?) constantly associated in the same chromosome with usually dominant ones would evade detection for generations and then becoming dominant might appear as reversions in a very confusing manner.

In their experiments on *Matthiola*, Bateson and Saunders¹⁵ mention two cases of correlated qualities which may be explained by the association of their physical bases in the same chromosome. "In certain combinations there was close correlation between (a) green color of seed and hoariness, (b) brown color of seed and grabousness. In other combinations such correlation was entirely wanting." Such results may be due to the association in the same chromosomes of the physical bases of the two characters. When close correlation was observed, both may be supposed to have dominated their homologues; when correlation was wanting, one may have been dominant and the other recessive. In the next paragraph to that quoted is the statement: "The rule that plants with flowers either purple or claret arose from green seeds was universal." Here may be a case of constant domi-

¹⁴ Bateson's term.

¹⁵ Bateson and Saunders, Experimental Studies in the Physiology of Heredity (Reports to the Evolution Committee, I., London, 1902) p. 81, paragraphs 11 and 12.

nance of two associated chromatin-entities.

Dominance is not a conception which grows out of purely cytological consideration. Cytology merely shows us the presence in a cell of two chromosomes, either of which is capable of producing some expression of a given character, and it is left to experiment in each case to show what the effect of this combined action will be. The experiment¹⁶ has shown that any one of the three theoretical possibilities may be realized, viz: (1) One or the other may dominate and obscure its homologue. (2) The result may be a compromise in which the effect of each chromosome is to be recognized. (3) The combined action of the two may result in an entirely new cast of character. In cases belonging to the first category, the visible quality (allelomorph, chromatin-entity) was described by Mendel as dominant and the other as recessive, and the experiments of Bateson and Saunders and others, as well as those of Mendel, have shown that in many cases a dominant character tends to remain dominant during successive generations if the environment is not materially changed. Nevertheless, some experiments cited by Bateson¹⁷ go to show that dominance may be variable or defective. Furthermore, it is not only conceivable, but highly probable that in most, if not all cases, there are many different expressions of each character (*i. e.*, many different allelomorphs as suggested by Bateson in regard to human stature), which on various combinations would necessarily exhibit relative dominance. The experiments with peas show an almost constant dominance of certain allelomorphs, such as round over wrinkled

in seeds, and of yellow over green in cotyledons; but it is worthy of note that here, as in most Mendelian experiments, only two antagonistic characters have been used. Investigations on varieties, in general similar, but exhibiting different expressions of some particular character, will certainly yield instructive results. Bateson's observations on crosses between single-, rose- and pea-combed fowls, represent a simple form of such a case and may be expected on completion to add much to our knowledge of the nature of dominance.

In addition to the many examples brought forward by Bateson in support of the Mendelian principle he cites three types of cases which are to be regarded as non-Mendelian. These are:

1. The ordinary blended inheritance of continuous variation.
2. Cases in which the form resulting from the first cross breeds true.
3. The "false hybrids" of Millardet.

1. *Blended Inheritance*.—In treating of this class Bateson clearly states the possibility that the case may be one entirely "apart from those to which Mendel's principles apply," but goes on to show how it may possibly be brought into relation with true Mendelian cases. He says in part: "It must be recognized that in, for example, the stature of a civilized race of man, a typically continuous character, there must certainly be on any hypothesis more than one pair of possible allelomorphs. There may be many such pairs, but we have no certainty that the number of such pairs and consequently of the different kinds of gametes are altogether *unlimited*, even in regard to stature. If there were even so few as, say, four or five pairs of possible allelomorphs, the various

¹⁶ Cf. Bateson and Saunders, *loc. cit.*

¹⁷ *Ibid.*

homo- and heterozygous combinations might, on seriation, give so near an approach to a continuous curve that the purity of the elements would be unsuspected, and their detection practically impossible." This hypothesis, which presents no difficulties from the point of view of the chromosome theory, is sufficient in the present state of our knowledge to bring many cases of apparently continuous variation into definite relation with strictly Mendelian cases; but, on the other hand, it seems probable, as already noted (p. 221), that the individual variation in many characters now thought to be strictly Mendelian may prove to be due to the existence in the species of many variations of what may be regarded as the type allelomorphs, accompanying similar variations of the homologous chromatin entities representing those types.

2. *First Crosses that Breed True*.—It is obvious that in the germ-cells of true-breeding hybrids¹⁸ there can be no qualitative reduction. In the normal process synapsis must be accounted for by the assumption of an affinity existing between maternal and paternal homologues, and conversely reduction is the disappearance of that affinity or its neutralization by some greater force. Now in *Hieracium* the characters of the hybrid are frequently intermediate between those of the two parents, showing that both allelomorphs (or chromatin-entities) are at work, but on self-fertilization there is no resolution of allelomorphs (reduction division). On the contrary, all the germ-cells are equivalent, as shown by the fact that all combinations produce similar offspring which in turn are similar to the parent. The suggestion made by Bateson in another connection, that "if one allelo-

morph were alone produced by the male and the other by the female we should have a species consisting *only* of heterozygotes," which would come true as long as bred together, at first sight seems logically applicable to these cases. For such an idea, however, we can find no cytological justification, since if any reduction occurs both chromosomes occur in both male and female germ-cells in equal numbers; and further, the evidence is in favor of a great variety of combinations of maternal and paternal chromosomes in the germ-cells so that the exact chromosome group of a hybrid parent could hardly be duplicated except by fusion of the very pair of cells separated by the reducing division. A more plausible explanation from the cytological standpoint is that the union of the chromosomes in synapsis is so firm that no reduction can take place, *i. e.*, that in each case, a paternal and a maternal chromosome fuse permanently to form a new chromosome which subsequently divides only equationally. The result must be germ-cells which are identical with one another and with those of the parents, and hence self-fertilization would produce offspring practically without variation. If this explanation be the correct one the process is distinctly pathological and hence it is not surprising that such cases, as noted by Bateson, should often present "a considerable degree of sterility."

3. *The "False Hybrids" of Millardet*.—Millardet, de Vries and Bateson have all described experiments in which the offspring resulting from a cross between dissimilar individuals showed the character of one parent only, those of the other parent being shown by further experiment to be lost permanently. The obvious cytological explanation of such a phenomenon is hinted at by Bateson in the

¹⁸ Cf. Mendel's experiments on *Hieracium*.

words "Such phenomena may perhaps be regarded as fulfilling the conception of Strasburger and Boveri, that fertilization may consist of two distinct operations, the stimulus to development and the union of characters in the zygote."¹⁹ Division of the egg without fusion of the pronuclei is a well-known phenomenon having been observed in eggs treated with chloral (Hertwig brothers) or ether (Wilson) and may be supposed to occur under certain unusual conditions in nature. In the experiments mentioned, however, both pronuclei continue to divide separately, while for a cytological explanation of the occurrence of "false hybrids" it is necessary to conceive not only the failure of the nuclei to copulate but the entire disappearance of one of them. Such a case would be comparable to that of chemically induced parthenogenesis or to the fertilization of enucleate egg-fragments, according as the nucleus remaining was maternal or paternal. Speculation in this connection, however, is unprofitable excepting so far as it may serve as a guide to research. A careful study of the fertilization of such cases as Millardet's strawberries, de Vries's *Oenothera* and Bateson's *Matthiola* crosses will no doubt be productive of immediate and positive results.

Mosaics.—A fourth class of non-Mendelian cases, the "mosaics" or "pie-balds" constitute a group in relation to which, as I believe, only negative evidence is to be expected from direct cytological study. A good example of the class is the "mosaic" fruit of *Datura* obtained by Bateson and Saunders, which, although in general exhibiting the thornless recessive condition, showed in exceptional cases a thorny patch. Of this case Bateson

says: "Unless this is an original sport on the part of the individual, such a phenomenon may be taken as indicating that the germ-cells may also have been mosaic." I must confess my failure to comprehend just what is here meant by mosaic germ-cells. I have attempted to show that in all probability the germ-cells are normally a mosaic of maternal and paternal chromosomes, but very evidently this is not Bateson's meaning.

From the standpoint of the chromosome theory I would suggest a possible explanation of the conditions as follows: We have already assumed that the somatic chromosome group, having a similar number of members to that of the cleavage nucleus and derived from it by equation divisions, is made up in the same way of pairs of homologous chromosomes. Every somatic cell, by this conception, must contain a double basis in the field of each character it is capable of expressing. In strictly Mendelian cases one of the homologues is uniformly dominant throughout the parts of the organism in which the character is exhibited. As already noted, however, it is unlikely that all the descendants of a dominant chromatin entity will be dominant. This is shown by the experiment of de Vries with sugar beets, which are normally biennial but always produce a small percentage of annual plants or "runners," which latter are regarded as recessives. The percentage of these runners may be increased by rearing the plants under unfavorable conditions and this is taken as evidence that the recessive allelomorphs may become dominant under such conditions.²⁰

If each cell contains maternal and paternal potentialities in regard to each character, and if dominance is not a

¹⁹ Bateson and Saunders, *loc. cit.*, p. 154.

²⁰ Cf. Bateson and Saunders, pp. 135, 136.

common function of one of these, there is nothing to show why as a result of some disturbing factor one body of chromatin may not be called into activity in one group of cells and its homologue in another. This would produce just the sort of a mosaic which Bateson and Saunders found in *Datura* or as Tchermak's pied yellow and green peas obtained by crossing the *Telephone* pea with yellow varieties. Correns describes the condition as *paeclodynamous* and his conception of the causes of the phenomenon as I understand it is parallel with that which I have outlined above. The logical possibility suggested by Bateson²¹ that the recessive islands in such cases as the mosaic pea may be due to recessive allelomorphs in the paired state does not accord with the theory of a chromosomal basis for those allelomorphs, since the chromosome groups, both of cells showing the recessive character and of neighboring cells showing the dominant one, are derived, so far as we know, by longitudinal or equation division from the chromosomes of the same original cleavage nucleus and hence must be alike.

The application of the theory here suggested may be put to test by an experiment in which hybrids of dissimilar true-breeding parentage are crossed and a third generation of "quarter-bloods" produced. Mosaics occurring in such an organism, if this theory be correct, would show one character resembling that of one of the maternal grandparents and one resembling that of one of the original purebreds of the paternal side. If both characters of the mosaic should be clearly paternal or maternal the theory as outlined is proven inadequate, since one of each pair of chromosomes, and hence the corresponding character-

group, is thrown out by the reduction-division in each generation.

In considering the behavior of the two chromosomes forming the basis of any given character, it was noted that in some cases the heterozygote character resulting from the combinations of dissimilar allelomorphs is sometimes totally unlike either of the latter. Thus Mendel found that in crosses between peas respectively 1 and 6 feet in height the offspring ranged from 6 to 7½ feet. In discussing similar cases, Bateson calls attention to the light which would be thrown on the phenomenon if we ventured to assume that the bases of the two allelomorphs concerned are chemical compounds; and he compares the behavior of the allelomorphs to the reaction of sodium and chlorine in the formation of salt. The results of chemical analysis show that one of the most characteristic features of chromatin is a large percentage content of highly complex and variable chemical compounds, the nucleo-proteids, and therefore if, as assumed in the theory here advanced, the chromosomes are the bases of definite hereditary characters, the suggestion of Bateson becomes more than a merely interesting comparison.

We have seen reason in the case of the true-breeding hybrids to suspect that the transmission by the hybrid of heterozygote characters may be due to permanent union of the homologous chromosomes. From this it is but a short step to the conclusion that even if, as is normally the case, the chromosomes do not fuse permanently, the very fact of their association in the same liquid medium may allow a possibility of a certain degree of chemical interaction. This must normally be slight, since its effects do not appear to be visible in a single generation; but the slightest of variation as a result of repeated new association, even though

²¹ Bateson and Saunders, p. 156.

it tends in diverse directions, must in time, guided by natural selection, result in an appreciable difference in a definite direction between a chromosome and its direct descendant and hence between the characters associated with them. In this we have a suggestion of a possible cause of individual variation in homologous chromosomes which we have already seen reason to suspect (pp. 221 and 226).

Finally, we may briefly consider certain observations which seem at first sight to preclude the general applicability of the conclusions here brought out. If it be admitted that the phenomenon of character-reduction discovered by Mendel is the expression of chromosome-reduction, it follows that forms which vary according to Mendel's law must present a reducing division. But the vertebrates and flowering plants—the very forms from which most of the Mendelian results have been obtained—have been repeatedly described as not exhibiting a reducing division. Here, therefore, is a discrepancy of which I venture to indicate a possible explanation in the suggestion first made by Fick²² and more recently by Montgomery.²³ This is to the effect that in synapsis as it occurs in vertebrates and other forms possessing loop-shaped chromosomes, the union is side by side instead of end-to-end as in Arthropods. In vertebrates, two parallel longitudinal splits, the forerunners of the two following divisions, appear in the chromosomes of the primary spermatocyte prophases. Both being longitudinal, they have been described as equation divisions, but if it shall be found possible to trace one to the original line of union of the two spermatogonial chromosomes side by side in synapsis,

that division must be conceived as a true reduction. A number of observations supporting this view will be brought forward in my forthcoming work on *Brachystola*.

Again, if the normal course of inheritance depends upon the accurate chromatin-division accomplished by mitosis, it would appear that the interjection, into any part of the germ cycle, of the gross processes of amitosis could result only in a radical deviation from that normal course. Such an occurrence has actually been described by Meves, McGregor and others in the primary spermatogonia of amphibians. In these cases, however, it appears that fission of the cell-body does not necessarily follow amitotic division of the nucleus. I would suggest, therefore, the possibility that the process may be of no significance in inheritance, since by the disappearance of the nuclear membranes in preparation for the first mitotic division, the original condition is restored, and the chromosomes may enter the equatorial plate as if no amitotic process had intervened.²⁴

There is one observation in connection with the accessory chromosome which deserves mention in any treatment of the chromosomes as agents in heredity. This element always divides longitudinally and hence probably equationally. It fails to divide in the first maturation mitosis, in which the ordinary chromosomes are divided equationally, but passes entire to one of the resulting cells. In the second maturation division, by which the reduc-

²² Fick, R., "Mittheilung ueber Eireifung bei Amphibien," *Supp. Anat. Anz.*, XVI.

²³ Montgomery, T. H., Jr., *loc. cit.*

²⁴ It is of interest in connection with this question that there occurs regularly in each of the spermatogonial generations in *Brachystola* a condition of the nucleus which suggests amitosis but which in reality is nothing more than the enclosure of the different chromosomes in partially separated vesicles. Cf. Sutton, W. S., "The Spermatogonial Divisions in *Brachystola Magna*," *Kans. Univ. Quart.*, IX., 2.

tion of the ordinary chromosomes is effected, the accessory divides longitudinally.²⁵

My observations in regard to the accessory chromosome lend support to the hypothesis of McClung²⁶ that of the four spermatozoa arising from a single primary spermatocyte, those two which contain this element enter into the formation of male offspring, while the other two, which receive only ordinary chromosomes take part in the production of females. If this hypothesis be true, then it is plain that in the character of sex the reduction occurs in the first maturation mitosis, since it is this division which separates cells capable of producing only males from those capable of producing only females. Thus we are confronted with the probability that reduction in the field of one character occurs in one of the maturation divisions and that of all the remaining characters in the other division. The significance of such an arrangement, though not easy of perception, is nevertheless great. As regards their chromosome groups, the two cells resulting from each reduction mitosis are conjugates and, therefore, opposites from the standpoint of any individual character. Thus if we consider a hypothetical form having eight chromosomes comprising the paternal series *A, B, C, D* and the maternal series *a, b, c, d*, one of the cells resulting from

the reduction division might contain the series *A, b, c, D*, in which case its sister-cell would receive the conjugate series *a, B, C, d*. It is plain that these conjugates, differing from each other in every possible character, represent the most widely different sperms the organism can produce. Now if reduction in the sex-determining chromatin also took place in this division it is apparent that these two diametrically opposite series would enter into individuals of different sexes; but if the sex-reduction is previously accomplished by the asymmetrical distribution of the accessory in the first division, then both the members of each conjugate pair must take part in the production either of males or of females and thus all extremes of chromosome combination are provided for within the limits of each sex.

POSTSCRIPT

The interesting and important communication of Guyer²⁷ on "Hybridism and the Germ-Cell" is received too late for consideration in the body of this paper. This investigator also has applied conclusions from cytological data to the explanation of certain phenomena of heredity, and his comparative observations on the spermatogenesis of fertile and infertile hybrids are an important contribution to the cytological study of the subject. The conclusions drawn are of great interest but, I think, in some cases, open to criticism. In assuming that there is a "segregation of maternal and paternal chromosomes into separate cells, which may be considered 'pure' germ-cells containing qualities of only one species" (p. 19), he repeats the error of Cannon which has already been

²⁵ The chromosome *x* of *Protenor*, which of all chromosomes in non-orthopteran forms most closely resembles the accessory, is also described by Montgomery (1901) as dividing in the reducing division, and failing to divide in the equation division—a fact which is the more remarkable because in *Protenor*, as in all Hemiptera-Heteroptera thus far described, reduction is accomplished in the first maturation division.

²⁶ McClung, C. E., "The Accessory Chromosome—Sex Determinant?" *Biol. Bull.*, III, 1 and 2, 1902. "Notes on the Accessory Chromosome," *Anat. Anz.*, XX., pp. 220-226.

²⁷ Guyer, M. F., "Hybridism and the Germ-Cell," *Bulletin of the University of Cincinnati*, No. 21, 1902.

dealt with in the early part of this paper. No mention is made in the paper of Mendel's law but in considering the inbred pigeon hybrids from which his material was obtained, the author expresses his familiarity with manifestations of the Mendelian principle by the statement that "in the third generation there is generally a return to the original colors of the grandparents." In cases which seem to resemble one grandparent in all particulars it is clear that the conception of pure germ-cells may be strictly applied, but the author was familiar with cases of inbred hybrids which plainly show mixtures. These he is inclined to explain in two ways as follows: (1) "Union of two cells representing each of the two original species would yield an offspring of the mixed type." (2) "Besides through the mixing just indicated, variability may be due also in some cases to the not infrequent inequalities in the division of individual chromosomes, through which varying proportions of the chromatin of each species may appear in certain of the mature germ-cells" (p. 20).

The first of these explanations would accord with the result of Mendelian experiment but for the fact that it is erroneously applied (and without cytological grounds) to *all* the characters or chromosomes instead of to individuals. As for the second passage quoted, there can be little doubt that irregular division of chromosomes would be likely to produce marked variation, but as Guyer himself observes, *these irregularities increase with the degree of infertility*. It seems natural to conclude, therefore, that they are not only pathological but perhaps in part the cause of the infertile condition. Furthermore, on the hypothesis of individuality of chromosomes, which Guyer accepts, the loss of a portion of a chromosome by irregular

division would be permanent and the effect of repetitions of the operation upon the descendants of a single chromosome group (which he regards as transmitted as a whole) would be so marked a depletion of chromatic substance as must lead soon to malfunction and ultimately to sterility.

As already noted (p. 216) the first of these two explanations of the causes of variation would allow only four possible combinations of chromosomes in the offspring of a single pair. But we know that except in the case of identical twins, duplicates practically never appear in the offspring of a pair however numerous the progeny. Therefore, whatever the number of the offspring, the variations of all except the few provided for by the four normal chromosome combinations must be accounted for by obviously pathological division processes, which tend strongly in the direction of sterility. But in the report of Bateson and Saunders to the Evolution Committee we find the statement: "We know no Mendelian case in which fertility is impaired" (p. 148). When we reflect that the vast majority of cases studied by these observers were Mendelian and connect this piece of evidence with the testimony of Cannon²⁸ that the maturation processes of variable cotton-hybrids are either normal or so distinctly abnormal as to entail sterility and with Guyer's own admission that the abnormalities in mitosis increase with the degree of sterility, the balance is strongly against the efficacy of pathological mitoses as factors in normal hybrid variation.

I take pleasure in acknowledging my indebtedness to Professor E. B. Wilson for invaluable counsel in the presentation of a subject offering many difficulties.

²⁸ Cannon, W. A., *loc. cit.*

Experimental Studies in the Physiology of Heredity

W. BATESON and R. C. PUNNETT

Reprinted from *Reports to the Evolution Committee of the Royal Society*, Reports 2, 3, and 4, 1905–08.

One of the first questions to arise from a study of Mendel's experiments on peas is whether or not the demonstrated independence of inheritance always holds true. In the series of experiments described in these papers Bateson and Punnett show that neither the inherited particles (which we now know as genes) nor the characteristic expressions of these particles always show independence. These papers are the first of a long and continuing series that show the geneticist how to derive information about the actions of the genes through careful study of the kinds and proportions of the phenotype, that is, the visible expression of the gene action in the characteristics of the organism.

Bateson and Punnett experienced great difficulty in the analysis of their experimental results. For one familiar with the work, the interpretation is obvious, but it must be remembered that these men were pioneers in virgin territory. In each of their reports it will be easier to understand just what has taken place by ignoring the conclusions the authors draw, and investigating the experimental results. The data themselves show the way to the reader, and attempts to follow the authors' lead result in confusion and misinterpretation. It is not a common occurrence to find that an experiment points the way, but the author does not follow the arrow. It does sometimes happen, however, and should always be looked for by a reader. One cannot challenge an author's data except through repetition of his experiments, but his conclusions are always subject to the tests of analysis and logic.

In the analysis of the combs of poultry, Bateson and Punnett use a terminology entirely their own. It is not unusual to have several symbolic systems during the early development of a field, with all but one eventually dropped. This particular experiment, with conventional symbols, has been used in many if not all genetics texts, but will be unfamiliar to almost everyone in Bateson and Punnett's language. One of the challenges in reading the original literature, however, lies in the fact that no one but the original author is placing an interpretation on the material.

The poultry paper is important in that it demonstrates the inde-

pendence of particle (gene) inheritance, although the final expression of the two independent pairs of genes depends upon an interdependent action. All we have learned of the mode of gene action since the time of this paper indicates that this is by far the most common situation, and that completely independent action of a pair of genes at a single locus is rare indeed. It is perhaps unfortunate that most people whose knowledge of genetics comes from high school courses or a general university course are exposed principally to the atypical results of Mendel rather than to an analysis of the mode of action of the gene.

In their original paper Bateson and Punnett included eight pages of data on their experiments. I have deleted most of these tables, and have retained only those experiments necessary for the analysis (Table 1). I have removed all notes on the extra toe character, which are not pertinent to our analysis. I have added an extra column to those of Bateson and Punnett so that the reader can test his understanding of the experiment by filling in the mating as symbolized by conventional methods.

The first part of the poultry paper includes the authors' interpretation of their results. Careful reading will show that it is almost completely erroneous. This was realized by the authors, and the second part of the paper comes from a later volume of the Reports. Here the authors correct themselves, and re-evaluate their results. Again the symbolism is likely to be confusing, but the Punnett square on p. 51 becomes more clear if the reader understands that "no P" or "no R" actually refers to the occurrence of a recessive allele, such as "p" or "r."

Bateson and Punnett's work on the sweet pea is significant for several reasons. The paper demonstrates first of all that heredity in the sweet pea is considerably more complicated than it appeared in Mendel's work with a closely related species, the garden pea. It also demonstrates that the independence of passage of the genes between generations can be lost. Again, it is necessary to inspect the authors' data, and not to allow their conclusions to lead to confusion. The authors appear anxious to have their results express some general rule through the occurrence of significant proportions, as was the case with Mendel's experiments. Their first example, utilizing the characteristics of height and growth pattern, is typically Mendelian, with independence of the genes both in their mode of transmission and in their expression. The second example, utilizing the hooded standard and flower color, is similar to that of the poultry, in that it shows interrelationships between two sets of alleles in the formation of a specific expression. Their third example is the most significant for our purposes. Their use of the term "partial gametic coupling" as an explanation of the situation they found with pollen shape and flower color does not, of course, explain anything. It is a typically human failing to feel that one understands something when one has been able to name it. Bateson and Punnett do not make this mistake, for, although they assign names to each situation as it arises, they

acknowledge on page 59 that "we have not perceived any circumstance to which the distinction can be attributed." I have noticed that my students are often satisfied with a name and do not always require an explanation as well.

The clue to understanding this paper lies in the data presented in the table on page 58. These data show that both pairs of genes are completely independent in themselves, and show results to be anticipated under the laws of chance. When the results are combined, however, the data deviate so greatly from that expected in the independent assortment of units that it is necessary to conclude that the independence has been lost. Bateson and Punnett do not satisfactorily explain this loss, although information in the literature would have enabled them to do so. Sutton's 1902 paper (page 27) suggested that there must be more than a single gene on each chromosome as a consequence of the greater number of characteristics than chromosomes. Sutton, however, could not prove this conjecture. Bateson and Punnett have the data which do so, but did not recognize this fact. The need to keep up with the current literature is a paramount one in biology. The data demonstrate most satisfactorily the existence of non-independent loci, which we now call "linked," and the existence of crossing over between the loci through the exchange of chromosome parts, for their data show that the loci can be separated.

POULTRY

$\frac{R}{2}$ is extracted recessive in F_2 .

THE FOLLOWING TABLE SHOWS THE results obtained respecting comb characters and extra toes. The numbers prefixed to the experiments enable the reader to trace the relationships in the case of all cross-bred birds. To save space the relationships of the pure-bred birds are not given. No case was seen in which this was found to influence the result. Slight and equivocal indications of a change of "prepotency" due to in-breeding were mentioned in Report 1. Fuller experience strongly suggests that these appearances were due to accidental fluctuations.

In the tables, DR is $F_1, \frac{DR}{2}, \frac{DD}{2}$, are,

The asterisk (*) means that the bird had some special origin, which can be ascertained from the breeding. R^* , for instance, may mean R from $DR \times R$, or R appearing in F_1 (see Report 1, p. 116), etc. These distinctions must be indicated, but they do not seem to affect the results.

p.p., r.r., mean that the bird was proved to be pure pea or pure rose; i.r., i.p. and p.s., that it was proved to contain single.

Doub. means the longitudinally split single, described later.

r.p. is the rose-pea, or "walnut" comb, whether natural (Malay) or artificial.

The same bird is given as DD in its matings with a single, and as r.r. or p.p., in its matings with one of the dominants.

respectively, heterozygous and homozygous dominants in F_2 .

TABLE I

Experiment Number	Female Number Comb type	Male Number Comb type	Nature of Mating	Combs of Offspring r.p. r. p. s.	Conventional symbolism for mating
90	91 p.	Br.I. s.	DR × R	12 21	
103	440 r.	435 s.	$\frac{DD}{2} \times R$	36	
105	4 s. birds	608 s.	R × R	131	
110	644 p.	419 p.	DD × i.p.	25	
112, 113A, 114	3 r. birds	558 s.	DR × R	67	
113	511 r.	558 s.	$\frac{DD}{2} \times R$	61	
121	530 r.	544 r.	$\frac{DR}{2} \times \frac{DD}{2}$	36	
122	372 s.	544 r.	$R \times \frac{DD}{2}$	39	
123	683 p.	544 r.	$p.s. \times \frac{rr.}{2}$		
123A	514 r.	544 r.	? × RR	6	
124	2 birds	r.	r.s. × p.s.		
125	4 birds	p.	DR × DR	43 16	
134, 135, 137	3 birds	p.	$p.s. \times \frac{rr.}{2}$		

TABLE 1—Continued

Experiment Number	Female Number Comb type	Male Number Comb type	Nature of Mating	Combs of Offspring			Conventional symbolism for mating
				r.p.	r.	s.	
136	527 s.	461 r.	$R \times \frac{DD}{2}$		21		
156	3 birds	984 r.	$\frac{?}{2} DR \times \frac{DR}{2}$		51	24	
157	440 r.	984 r.	$\frac{DD}{2} \times \frac{DR}{2}$		5		
162	881 r.p.	904 r.p.	r.p. \times r.p.	4			
163-166A	5 birds	904 r.p.	s. \times r.p.	51	44	39	47
167-170	4 birds	842 r.p.	s. \times r.p.	35	32	32	37
171-172	3 birds	842 r.p.	r.p. \times r.p.	56	17	23	13
173, 175	2 birds	855 r.p.	r.p. \times r.p.	38	10	16	4
174, 176, 176A-B	4 birds	855 r.p.	s. \times r.p.	53	66	41	57
184	527 s.	461 r.	$R \times \frac{DD}{2}$		9		
185, 187	2 birds	461 r.	p.s. \times $\frac{rr.}{2}$	30	20		
186	664 p.	461 r.	p.p. \times $\frac{EE}{2}$	10			
188-191	5 birds	Mal. r.p.	s. \times r.p.	22	21	20	20

DISCUSSION OF RESULTS

Comb-characters

DR birds, r. or p., produced in various ways, bred with R birds (s.c.) of various origins gave 449 D to 469 R. DR \times DR gave a total of 211 D to 83 R. These totals¹ are not very far from the 1 D : 1 R or 3 D : 1 R severally expected. Individually, however, there are some wide departures from these expectations, and it is practically certain that in several cases there was distinct numerical inequality of D and R gametes, as seen in the case of Peas and elsewhere. On the other hand there is no indication that DR individuals themselves are capable of subdivision into classes, and all the figures available point to a monomorphic distribution of the aberrant individuals round the mean case of equality in output of D and R. There is also distinct evidence that the proportional output of D and R may change at different times in the life of the same individual. There is, as yet, no clear case, unfortunately, in which a DR ♂ giving an aberrant number with one s.c. ♀ was simultaneously bred with another s.c. ♀. It is practically certain, nevertheless, that the irregularity did not, in any way, depend on the recessives, for the same bird often gave regular numbers with 1 DR and aberrant numbers with another. In these comments it is assumed that no gametic selection occurs.

"Extraction" had no influence on the proportions, as may be seen from the table.

An example of a
♀ extracted pure
dominant (rose)
occurs in Experiment 103 and 157

¹ They include chicks kindly bred by Mr. Assheton (not given in the table) from birds raised in Experiment 33, as follows: DR \times R, 16 r. 6 s. DR \times DR, 36 r., 16 s.

Another, ditto . . .	"	113
An example of a ♂ extracted pure dominant (rose)		
occurs in	"	121—123A
Another, ditto . . .	"	134—137
Another, ditto	"	184—187

Very few F₂ p.c. birds have been tested, and, as it happens, no case of pure extracted dominant F₂ has been met with, but ♀ 644 from DR \times D was, however, a pure DD (Experiment 110).

Purity of Recessive (Single) Comb.—Singles of various origins, derived in several ways from rose and from pea crosses, when mated together, have never given either rose or pea. In all, such matings have given 800 singles. In one case (Experiment 105) *four specimens of a new form of comb* appeared, together with 131 singles (included in total just given).

This mating was made of 4 F₁ recessive ♀'s from a "non-Mendelian" or mixed F₁ from rose-comb, bred with a similar F₁ recessive ♂ from pea-comb. In the four mutational birds the combs were imperfectly split posteriorly (see p. 113). These must be regarded as illustrations of that twinning or duality which has been observed in so many median organs. A similar case of posterior duality in a rose-comb² came in Experiment 166. Such double "single" combs are not very rare in fowls, but are not found in any recognized breed, the Houdan "leaf" comb being, in some ways, distinct.

Relation of Rose to Pea Comb.—In 1902 this inquiry was begun by crossing DR rose-combs with DR pea-combs (Experiment 124). These birds may respectively be called r.s. and p.s. They gave, as was expected, four

² In 1904 a case of partial duality in a pea-comb has occurred. A split s.c. like the mutational form occurred also in Experiment 176A.

types of comb, viz., s.s. singles, r.s. roses, p.s. peas, and finally r.p. combs, the latter being a type not before seen in the course of the work.³ It is characteristic of their comb that it is wide like a rose, though much less papillose and with no posterior point or "pike"; but the most singular feature of it is the presence of small bristles or minute feathers on the posterior third. Often these feathers form a narrow band crossing the comb from side to side in front of the posterior third. Sometimes, however, there is no definite band, but the feathers are either generally distributed over the back or on the posterior sides of the comb. No such bristles or feathers ever occur on singles, roses, or peas. Their existence raises a morphological problem of some interest, but this cannot be treated as yet. In front of the feathered part of the comb is often a well-marked transverse groove. On approaching maturity the part anterior to the groove generally widens out (especially in males) and becomes corrugated, the posterior part remaining narrow and flat.

This peculiar comb is the structure known as the "walnut" comb, proper to Malays and to no other breed of fowls. As stated, it was here first formed indirectly by crossing r.s. \times p.s. and it has since been made directly by breeding both pure r. and extracted F₂ r.r. with pure p. Sometimes it is distinctly larger than in pure Malays, but the artificial walnut comb is often indistinguishable from the pure Malay type.

The results from the "artificial" walnut combs were as follows. Three such r.p. ♂'s were bred through the season

of 1903 with various s.c. birds (Experiments 163–170, 174, 176, 176A–B), and gave—

	r.p.	r.	p.	s.
139	142	112	141	+ 2 doubtful.
Simple expectation	133	133	133	133

Four "artificial" r.p. ♀'s with similar ♂'s gave (Experiments 162, 172 and 173, 175)—

	r.p.	r.	p.	s.
95	26	38	16	
Simple expectation	99	33	33	11

We have, therefore, in the first case a fair approach to equality, and in the second, figures not very far removed from 9 : 3 : 3 : 1. It is, therefore, clear that the artificial r.p. birds were giving off approximately equal numbers of gametes, r.p., r., p., s. The individual results show some irregularities, especially 176B, which gave 10, 17, 7, 8, but the average result is unmistakable.

These facts show two points of interest, first, that some gametes are bearing r.p. unsegregated; secondly, that s. is evidently present though it is presumed that in the parental gametes of the r.s. and p.s. birds the s. elements had been eliminated by segregation.

At first sight it seemed that the presence of s. indicated a failure of segregation. Further experiment, however, has shown that r.p. birds raised from pure r. and pure p. birds are in exactly the same condition as those raised from r.s. \times p.s. This latter observation, which will be described with the work of 1904, proves that s. gametes may in some way be formed by resolution of the product of the cross r. \times p.

The next step was to investigate the properties of the true Malay walnut, or natural r.p. comb, and especially to determine whether it could be re-

³ The features of the 3 forms of dominant were not satisfactorily distinguished in the earlier chicks from Experiment 124. They are therefore given collectively as 68 r., r.p., p. and 24 s.

solved into r. and p. Mr. Edgar Branford, of Woodbridge, who has a long experience of the breed, was kind enough to give much help in this matter, and further assistance was also received from Mr. Wootten, a breeder of White Malays. It appears that Malays bred *inter se* sometimes produce true peas as well as the normal walnut. Occasionally in certain strains peas come often, but generally speaking they are uncommon. Whether pure Malays ever throw actual roses or singles is not quite certain, but if they do, these occurrences must be excessively rare.

A pure black-red Malay ♂ and two pullets were obtained from Mr. Branford. The ♂ had been used by him in 1902 with pure hens, and certainly then gave no rose or single, though possibly occasional peas.

The Malay ♂ was bred with 5 s.c. hens of various origins and gave (Experiments 188–191)—

r.p.	r.	p.	s.
22	21	20	20

the equality of the four types of gamete being evident. The Malay hens are notoriously bad layers and only gave small totals. Each was bred with a s.c. cock and the result was

r.p.	p.
14	13, no true r.c. or s.c.

Of the pea-combs, 3 were of the high, intermediate type, inclining to s.c., but none was a true s.c. Of the r.p. group, 1 on hatching was regarded as an irregular rose, but it developed into a fairly ordinary type of walnut comb, though having two rather prominent knobs behind.

In Experiment 171 a Malay ♀ is recorded as giving × artificial r.p. ♂, 3

r.p., 1 r., 1 p., 1 s. The r. was irregular, and the s. was very low. In the light of later experience, it is perhaps possible that both were wrongly classified. Both were dead in the shell, and at that date the distinctions were not familiar to us.

In these results there are still certain points obscure. The "artificial" r.p. birds are giving off the four types of gamete. Therefore there must be five types of r.p. comb possible, namely r. × p., r.p. × r.p., r.p. × r., r.p. × p., r.p. × s.; but owing to the fact stated above, that s. may be created afresh by at least one of the combinations into which it does not directly enter, the subsequent analysis becomes very complex.

A further difficulty is suggested by the behavior of the natural r.p. Though the ♂ r.p. with s. ♀'s gave the four forms in equal numbers, it is practically certain that in Malay breeding, if r.c. and s.c are ever seen, they are excessive rarities; whereas if there were gametic equality on both sides, 3 in 16 should be r.c., and 1 in 16 should be s.c. For some time the conclusion seemed irresistible that the appearance of these forms on crossing with s. must be due to some imperfection of fertilisation, leading to a partial monolepsis and this account was provisionally adopted. At that time the difference between reciprocal crosses was not fully appreciated; but after a critical study of their material it is more likely that the true explanation lies in a difference of constitution between the ♂'s and ♀'s.

It is possible that only ♂'s with walnut combs produce all four kinds of gamete, r.p., r., p., s., other males giving only r.p., or r.p. and p., or r.p. and r.; while r.p. ♀'s give either r.p. and p., or r.p. only. If this conclusion is substantiated, it will be possible to give a complete account of this curious case.

POULTRY

Comb-characters

Relation of Rose-comb to Pea-comb

In the preceding Report we showed that, judged by the criterion of gametic output, 4 kinds of r.p. birds exist, viz., r. \times p., r.p. \times r.p., r.p. \times r., and r.p. \times p. Birds of the constitution r. \times p. gave, on mating with single comb, equal numbers of the 4 types r.p., r., p., and s. Birds of the constitution r.p. r. and r.p. p. gave when crossed with s. equal numbers of r.p. and r., and of r.p. and p. respectively, whilst a pure r.p. bird gave only r.p. when similarly crossed. We suggested that a fifth type of r.p. bird was conceivable, i.e., one giving off equality of r.p. and s. gametes. In order to test this possibility a pure r.p. ♀ was mated with a single comb ♂ (Experiment 268). Three ♀s. resulting from this cross were in the following year mated with their father (Experiments 342 and 343). From these matings all the 4 types of comb resulted. In other words, the gametic output of birds produced by the union of r.p. with s. cannot be distinguished from that of birds produced by mating r. with p. Judged, therefore, by gametic output, only 4 types of comb are known to exist.

Since the publication of the last Report we have been led to alter our views as to the nature of the r.p. comb, and have recently pointed out¹ that the case may be looked upon as one of simple dihybridism. On this view the allelomorphic pairs are (1) Rose and no-rose; (2) Pea and no-pea—the first member of the pair being in either case dominant to the second. The rose-comb is, therefore, in constitution Rose + no-pea (*R. no P.*) and the pea-comb Pea + no-rose (*P. no*

R.). Consequently the zygote formed by the union of r. and p. has the constitution *R. no P. : P. no R.*, and the appearance of the walnut form must be regarded as due to the presence in the zygote of the 2 unit characters *R.* and *P.* which belong to distinct allelomorphic pairs. From such a zygote 4 sorts of gametes must be produced in equal numbers, viz., *R.P.*, *R. no P.*, *P. no R.*, and *no R. no P.* Since the single on this view must be homozygous and producing gametes of the constitution *no R. no P.*, it follows that an *r.p.* bird such as we are considering will, when mated with a single, give rise to 4 zygotic types in equal numbers. Constitutionally these will be (1) *R. no R. : P. no P.*, (2) *R. no R. : no P. no P.*, (3) *P. no P. : no R. no R.*, and (4) *no R. no R. : no P. no P.*, and visibly such birds will be *r.p.*, *r.*, *p.*, and *s.* respectively. Again, when mated together, 2 such *r.p.* birds will produce *r.p.*, *r.*, *p.*, and *s.* birds in the ratio 9 : 3 : 3 : 1. The constitution of such birds is set forth in the accompanying table (p. 13), which shows that the r.p. birds may be of 4 sorts, viz. (1) homozygous for both *R.* and *P.*; (2) homozygous for *R.* and heterozygous for *P.*; (3) homozygous for *P.* and heterozygous for *R.*; and (4) heterozygous for both *R.* and *P.* The relative proportions of birds belonging to the 4 classes (1), (2), (3), and (4) will be 1 : 2 : 2 : 4. As the table shows, one of the 3 roses and one of the peas will be homozygous.

We have already pointed out² that the proportion of the different combs in the various matings affords no criterion for judging between this view and the one previously suggested. The new view, however, explains the identity of the combs produced by the crossing of r. with p. and of r.p. with

¹ Proc. Camb. Phil. Soc., 13, p. 167.

² Loc. cit.

RP RP (r.p.)	RP R, no P (r.p.)	RP no R, P (r.p.)	RP no R, no P (r.p.)
R, no P RP (r.p.)	R, no P R, no P (r.)	R, no P no R, P (r.p.)	R, no P no R, no P (r.)
no R, P RP (r.p.)	no R, P R, no P (r.p.)	no R, P no R, P (p.)	no R, P no R, no P (p.)
no R, no P RP (r.p.)	no R, no P R, no P (r.)	no R, no P no R, P (p.)	no R, no P no R, no P (s.)

s., thus dispensing with the conceptions of compound allelomorphs and synthesis.

During the past two years we have tested a considerable number of r.p. combs, with the result that each of the 4 types has been met with over and over again. Speaking generally, this form of comb exhibits much variation in size, shape, and amount of the special feathering on its posterior surface, though hitherto we have been unable to establish any connection between such variations and the gametic output of individuals in which they occur. We may now consider in rather more detail the matings in which the various types of r.p. combs are concerned.

Pure r.p. (zygotic constitution *R.P.R.P.*).—This type has occurred in 2 Malay bantam hens (Experiments 242, 268, 284) and in a black-red Malay ♂ (Experiment 351) all purchased. It has also occurred in F_2 from an original cross of r. \times p. (Experiments 255, 256, 333, 383). Further, we have one case (Experiment 366) where it was formed from 2 r.p. birds which were produced by the mating of r.p./p. with single. Since every bird with an r.p. comb must give off some gametes at any rate containing both *R.* and *P.*, we may, of course, expect some pure r.p. birds to be produced by the mating of any 2 birds with this form of comb. In all

the above experiments r.p. combs alone resulted from the union of the pure r.p. bird with a single.

Combs r.p. \times p. (zygotic constitution *R.P.P. no R.*).—(Cf. Experiments 196 to 197, 239, 271, 278, 287, 305, 314, 328, 336, 355, 357 to 358, 385, 387 to 388, 390, 391.)

The birds tested in Experiments 196 to 197, 239, and 287 were Malays with natural r.p. combs. The rest were produced artificially in F_2 from original matings r. \times p., r.s. \times p.s., or r.p. \times s. On mating with singles this type of r.p. comb gave in all 285 r.p. and 296 peas, the expectation, of course, being equality.

Combs r.p. \times r. (zygotic constitution *R.P.R. no P.*).—(Cf. Experiments 233, 283, 297, 316, 338 to 339, 360, 371 to 372.)

The bird tested in Experiment 283 was a ♂ Malay bantam, whilst those in Experiments 233 and 316 were Malay ♀ s. The rest were artificially produced. Altogether 289 birds were produced, of which 149 had r.p. and 140 rose-combs, expectation as in the last case being equality.

Combs giving all 4 with Single (zygotic constitution *R. no P. P. no R.*).—(Cf. Experiments 208, 212 to 214, 217 to 218, 232, 234, 236, 238, 240 to 241, 243, 249, 251 to 252, 260 to 262, 272, 275, 277, 298, 300, 302 to 303, 306

to 307, 309, 311 to 313, 315, 317 to 320, 323 to 327, 329, 331 to 332, 334, 342 to 343, 345 to 348, 359, 361 to 365, 373 to 374, 382, 386.)

Of the 1,251 birds produced in the above experiments 373 were r.p., 425 pea, 413 rose, and 440 single, expectation in each case being 413. There is a marked deficiency of r.p. and a distinct excess of singles. These disturbances in the expected ratio of equality we are inclined to attribute to the behaviour of the 4 ♂ s. used in Experiments 306, 347 to 349, and 373 to 374—cases which must be considered in rather more detail.

The ♂ s. in Experiments 306 and 349 gave only 10 r.p. birds out of a total of 93, i.e., only half the number expected, although the total number of chicks hatched was fairly large. These cases were not followed up, but when the same phenomenon reappeared in Experiment 373, the ♂ bird was mated also to 4 pure Br. L. ♀ s. (Experiment 374). Again, there was a marked deficiency of r.p. chicks, pointing to something unusual happening among the gametes of this ♂. The bird has been kept in order that further experiments may be made next year.

Experiments 347 to 348 seem to point to a disturbance of a somewhat different nature. The ♂ (No. 144 of

1904) was originally mated with 3 single combed ♀ s. to test the nature of his comb. Later on a black Andalusian ♀ and 2 Wh. L. ♀ s. were introduced. The ♂ turned out to be giving off all 4 kinds of gametes, giving with the 3 original ♀ s. and the black Andalusian 18 s., 26 r., 26 p., and 25 r.p. With the 2 White Leghorn hens, however, he gave an extraordinary deficiency of r. and r.p., the numbers being 17 s., 2 r., 17 p., and 3 r.p.

In the case of each of these 4 birds the numbers are so aberrant that we can for the present hardly avoid regarding them as due to some disturbing circumstances. The number of chickens involved is 309, viz., 107 s., 75 r., 86 p., and 41 r.p. Subtracting these numbers from the sum totals given above, we are left with 333 s., 338 r., 329 p., and 332 r.p., which is a very close approach to the expected ratio of equality.

Rose Pea-combs Mated Together.

Zygote R. no P. P. no R. × zygote R. no P. P. no R. In the last Report (p. 110) were mentioned four experiments of this nature in which the figures obtained were not far removed from the expected ratio of 9 : 3 : 3 : 1.

Further experiments of the result of this type of mating were made in 1904, and are set out in the table below:

	s.	r.	p.	r.p.
Experiment 201	2	5	7	16
" 202	2	5	9	8
" 204	10	5	4	15
" 205	2	9	8	22
" 206	2	4	5	9
" 215	4	19	19	33
" 216	2	4	5	11
" 244	—	—	2	2
" 245	1	5	4	13
" 253	6	8	14	36
" 254	3	5	9	12
Expectation	34 22.9	69 68.7	86 68.7	177 206.1

The result discloses a distinct excess of singles and peas, together with a deficiency of r.p.s. Examination of the table shows that whilst the result in certain cases (e.g., Experiments 201, 205, 216, 245) fits closely with expectation, it is in other cases far removed from it. Thus in Experiment 204 we should have expected not more than 2 singles, whereas the actual number is 10. Yet the subsequent Experiments 212 and 300 prove that the ♀ was then giving off all four sorts of gamete in equal numbers, whilst Experiments 208 and 303 show that the same was also true of the ♂. It is conceivable, of course, that the numbers in Experiment 204 may be the result of chance, but, having regard to the fact that wherever any serious disturbance of the ratio 9 : 3 : 3 : 1 occurs it is generally accompanied by both excess of singles and deficiency of r.p.s., we are more inclined to attribute such aberrations to the presence of some disturbing circumstance hitherto undetermined.

*"Extracted" Combs from Mating
r.p. × r.p.*

Singles "extracted" from the cross r.p. × r.p. were mated together in Experiments 209, 210, 247, and 248. Of the 144 chicks resulting, all were single.

Seven extracted roses were tested, with the result that one turned out to be homozygous (Experiment 273) and the rest heterozygous (Experiments 246, 263 to 266, and 274). With single combed ♂ s. these 6 birds produced 176 chicks, of which 92 were single and 84 rose. Extraction has clearly no influence upon the behaviour of rose and single combs.

An extracted ♂ pea-combed bird produced (in Experiment 230) 2 singles and 3 peas with a Br. L. ♀. In Experiment 231 an extracted pea-combed ♀ gave, with an s.c. ♂, 5

singles and 8 peas. In Experiment 228 an extracted p.c. ♂ was bred with 3 extracted p.c. ♀ s., and gave 20 singles to 34 peas, the expectation being 13·5 : 40·5. Such an aberration from the expected ratio may easily be the result of chance, though it is possible that it may be due to the prevalence of intermediate pea-combs.

Intermediate Pea-combs.—In certain families where the pea-comb was concerned, heterozygote (p.s.) birds occurred with some frequency showing so little of the pea character that as young chicks they could easily be taken for singles. The median ridge is very high, approaching the single comb, whilst the lateral ridges are much reduced. Such a comb requires very close scrutiny in the newly hatched chick to distinguish it from a single. In one case such a bird, recorded as a single when a chick, eventually grew up with one of these intermediate pea-combs. Experiments with reference to the nature of this comb are still in progress, and until they are complete we propose to defer our detailed account. We have alluded to the case here because it may have some bearing on the excess of singles in Experiments 231 and 357. At the time of that experiment we had not fully grasped the distinction between the intermediate pea and the single comb, and it may very well be that some of the combs then recorded in certain families as single were in reality of the intermediate type.

The Malay Comb

In the last Report (p. 111) we drew attention to the paradox that real rose and single combs are not known to occur among Malays when bred pure, and we suggested as a possible explanation that the ♂ s. only are capable of producing all four forms of gamete. In a subsequent note (p. 112) we alluded to a ♀ Malay which was re-

corded to have given (with a single combed ♂) 2 s.c., 1 r., 1 p. and 1 r.p. This ♀ was again tested in 1905 (Experiment 334) and gave with a Br. L. ♂ 7 s., 2 r., 2 p., and 3 r.p. There is, therefore, no doubt that ♀ Malays as well as ♂ s. can give off all four kinds of gamete. Unfortunately we were unable to procure a ♂ Malay of this nature in 1905, and the absence of roses and singles in pure Malays must, for the present, remain unexplained.

SWEET PEAS *Bush* × *Cupid* Cross

The Bush Sweet Pea, a form not previously used in these experiments, has a peculiar conformation. After reaching a height of about 8 inches it branches profusely from its lower nodes. Of the stems thus formed about 10–15 grow on, eventually reaching a height of some $3\frac{1}{2}$ feet. The production of branches continues, but those formed later remain short and slender. In an ordinary tall Sweet Pea only about four or five stems attain any considerable extension (without special treatment). The stems of the Bush are thin and rather wiry, especially at the base, and the plants, till they reach a height of about 18 inches, can almost maintain themselves erect without sticks. By that time the stems are mutually entangled by their tendrils, and the compact bush-like form is very pronounced, entirely distinguishing them from any other sort of Sweet Pea.

In both the ordinary tall plants and the ordinary Cupids there appears to be some factor which restrains the production of branches, and thus enables the plant to attain a greater length. The non-branching tall plant attains a height 2 or 3 feet greater than that of the Bush, which must be regarded as a branching "tall." Sim-

ilarly, the erect or branching Cupid is more stunted in its length than the ordinary or non-branching Cupids. The allelomorphs concerned are evidently:—

	Dominant	Recessive
1. Tallness (T)		Dwarfness (t)
2. Prostrate: non-branching (P)	Erect: branch- ing (p)	

The ordinary tall is $TTPP$, the prostrate Cupid is $ttPP$, and the Bush is $TTpp$. When Bush is crossed with Cupid the two complementary factors, T and P, necessary to the production of the full height, meet each other and the "reversion on crossing" occurs.

In F_2 , besides the three forms already mentioned with which we were familiar, there appears also, in consequence of the recombination of the factors, a new type—the erect dwarf ($ttpp$). These little plants have a very singular appearance, being extremely short and erect, somewhat resembling box-edging.

The numbers in the F_2 generation from six F_1 plants were:

	Observed	Calculated
Tall (= prostrate talls)	219	217.8
Bush (= erect talls) ..	70	72.5
Prostrate Cupids	69	72.5
Erect Cupids	29	24.2
	387	387

The observed numbers approach closely to those calculated on the 9 : 3 : 3 : 1 ratio, and the case is evidently one of dihybridism in which the two pairs of factors concerned are those we have suggested above.

The families resulting from this

TABLE I

—	Tall	Bush	Cupid prostrate	Cupid erect	
Purple {long	54	18	10	1	83
erect {round	4	—	1	1	6
Purple {long	24	11	5	8	48
hood {round	—	—	—	—	—
{long	3	2	—	—	5
Red .. {round	23	11	6	2	42
White {long	43	10	10	3	66
erect {round	11	2	1	3	17
White {long	20	5	4	1	30
hooded {round	—	—	—	—	—
Colour and pollen	37	11	32	10	
Total	219	70	69	29	
Expectation	217.8	72.6	72.6	24.2	

* Owing to late germination and consequent failure to flower.

cross are considered elsewhere (p. 12) in connection with gametic coupling.

The Inheritance of the Hooded Character

The work of the past two seasons has consisted largely in the investigation of the peculiar transmission of the hooded standard. The hood is constituted by a more or less pronounced folding downwards of the top and sides of the standard or vexillum³ (*cf.* Fig. 1). The hooded varieties may be of almost all the colours known in Sweet Peas—white, cream, red, pink, mauve, purple—but so far as we have observed, the hood never occurs in the purple bicolor or Purple Invincible (P. I.), or in the corresponding red bicolor or Painted Lady (P. L.). In all the hooded types standard and wings are more alike in tint; and there is therefore some interdependence be-

tween colour and form such that either the bicolor character prevents the development of the hood, or the development of the hood modifies the colour.⁴

⁴ The purple hooded types produced in these experiments are those known as Duke of Westminster and Duke of Sutherland respectively. The former corresponds with P. I., and the latter is represented among the erect types by the purple with purple wings referred to in these Reports as P.p.w.

The colour difference between hooded and flat types is not easy to express, but may readily be perceived when a collection of Sweet Peas is examined. In the erect purple and red bicolors the colour of the standard contains a red quality distinguishing it from that of the wings, which are more blue in the case of purples and more pink in the case of reds. While in a purple bicolor the standard is a chocolate red and the wings are blue or purple, the corresponding hooded type has both parts purple. Similarly in a red bicolor the colour of the standard is a scarlet and that of wings pink or pinkish-white, while in the corresponding hooded type the pigment of both parts is pink. When, as sometimes happens, the standard and wings of a hooded type differ in colour, the difference is rather in the amount of pigment than in its quality, the standard being fuller and the wings lighter, but the pigments of both parts show little difference in tint.

³ This shape is associated with, and perhaps caused by, absence of the central notch which is conspicuous in the middle of the erect standard of old-fashioned flowers. The shape of the buds in the two types is consequently very distinct (see figures).

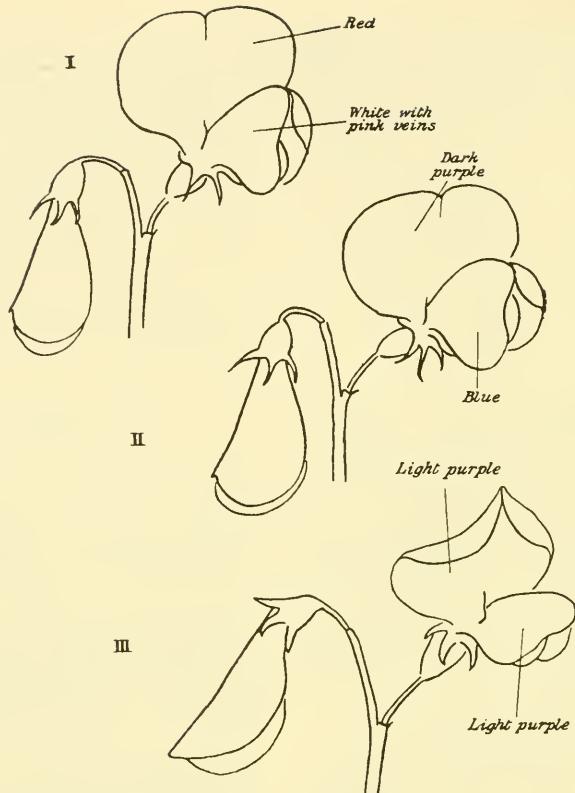


Fig. 1. Diagrammatic representation of the three coloured types which occur in F_2 from Blanche Burpee (hood white) \times E. Henderson (erect white), giving F_1 purple erect. I. Painted Lady; II. Purple Invincible; III. Duke of Westminster.

The erect type of standard is dominant to the hooded type.

The original cross with which we started was made in 1904 between the two whites, Blanche Burpee (long pollen, hooded) and Emily Henderson (round pollen, erect standard). The majority of the F_1 plants were P. I.

(some being whites, others P. L., according to the factorial composition of the parents), and it is from such plants and their offspring that the data put together in Table II were obtained. From the four F_1 plants 773 plants were raised in F_2 , with the following results:

Erect purples	Hooded purples	Erect reds	Whites (erect and hooded)
232	83	112	346
$\brace{315 \text{ [326]}}$		$\brace{[109]}$	
$\brace{427 \text{ [435]}}$			[338]

As was to be expected, the purples are three times as numerous as the reds,

and the coloured are to the whites as 9 : 7. From the F_2 generation families

were raised in F_3 and F_4 , as is recorded in Table II. The striking feature about this series of experiments is that among the coloured flowers hoods are only found on the purples. Among some thousands of plants, not a single hooded red has appeared. Nevertheless, the existence of hooded red types is well known in Sweet Peas. We are therefore led to suppose that we are dealing with a strain in which the factor for erect standard is alternative in the gametes to the factor for blue; and, conversely, that every gamete which carries the factor for the erect standard

is devoid of the blue factor. Consequently, for these two pairs of factors, only two of the four conceivable classes of gamete exist, viz., blue hooded gametes, and non-blue erect gametes. The spurious allelomorphism (see p. 4), which we must assume to exist between blue and hood on the one hand, and between non-blue and erectness on the other, does not allow of the formation of purple erect, or of red hooded gametes. If this assumption is correct, there should follow certain consequences which may be tested by the data given in Table II.

1. Every hooded purple must be homozygous for the blue factor.
2. Every red must be homozygous for the erect standard.
3. Every erect purple must be heterozygous for both the erect standard and for blueness, and must therefore give hooded purples, erect purples, and reds, in the ratio $1 : 2 : 1$.
4. Every hooded white must be homozygous for the blue factor.
5. Since every hooded plant is homozygous for purple, and since long pollen is partially coupled with the blue factor, it follows that round pollen should be much rarer among the hooded than among the erect purples.

The phenomenon of association of hood with purple was also witnessed in the F_2 families resulting from the Bush \times Cupid cross, with which we have already dealt in another connection (p. 2). Here, again, the hood occurred in F_2 , and only on the purples. The numbers—48 hooded purples, 89 erect purples, and 47 erect reds—are very close to the expected ratio, $1 : 2 : 1$.

As stated above, in other strains red

In Experiments 48–53, six hooded purples, although from families in which reds occur, bred true to purple (except in so far as whites might appear).

In Experiments 54–59, six reds were bred from, and proved to be all homozygous for the erect standard.

The erect purples, whose offspring are recorded in Experiments 1–47, all gave hooded purples and erect reds. Of their 3707 coloured offspring, 902 were hooded purples, 939 were erect reds, and 1920 were erect purples.

In the only two cases in which the cross between a hooded white and a red has succeeded, the coloured offspring have been purple. We hope to obtain further results in the present year.

So far only two round hooded purples and one round hooded white have been met with in families heterozygous for hood and purple (Experiments 1–47). With this point we shall deal later, in connection with partial coupling (see p. 13).

hooded forms exist. Experiments with these types are in progress, but have not yet been carried far enough to give positive results.

Partial Gametic Coupling

A. *Between Pollen and Colour*.—In our last Report, we showed that the distribution of pollen characters, long grains and round grains, was affected by that of the colour characters, according to a definite system, and we

pointed out that the experimental results were explicable on the assumption that in plants, heterozygous for colour and pollen, the gametes were produced in a series of 16, viz., 7 purple long, 1 purple round, 1 red long, and 7 red round. Purple is associated with long pollen and red with round, but in neither case is the coupling complete.

In the four F_1 families from the cross *Blanche Burpee* \times *Emily Henderson* (Table II, Exps. 1–4), the distribution

From one of these F_2 families (No. 2), ten erect purples were chosen and

of the pollen fits in with this scheme, as the following numbers show:

	Observed	Expectation on the 7:1:1:7 basis
Purple long ...	296	295
Purple round ..	19	25
Red long	27	25
Red round	85	82
	427	427

grown on in the following year (Nos. 5–14). They produced:

	Purple long	Purple round	Red long	Red round
F_3 generation from No. 2	493	25	25	138
Expectation on 7:1:1:7 basis	471	40	40	130
Expectation on 15:1:1:15 basis	490	20	20	151

The number of round purples and of long reds is in each case decidedly lower than would be looked for on the assumption that the coupling was on a 7:1:1:7 basis, and is much nearer to what would be expected if the coupling were on a 15:1:1:15 basis. In

some families, the dearth of these two classes is particularly well marked, and two of them (Nos. 5 and 6) were bred from. From No. 5, five families (Nos. 15–19) were obtained, and these, with the present plant, gave the following result:

	Purple long	Purple round	Red long	Red round
No. 5 and its offspring, Nos. 15–19	545	25	40	159
Expectation on 7:1:1:7 basis	532	45	45	147
Expectation on 15:1:1:15 basis	554	23	23	169

The results are irregular. The proportion of red longs to red rounds fits fairly well with the supposition that the coupling is on a 7:1:1:7 basis; but the purple rounds are only half as many as would be expected. The coupling between long and purple seems to be closer, and is in accordance with the supposition that the gametic series

is 15:1:1:15. Both in the F_3 generation and in these F_4 plants there is evidently some disturbing process which we cannot at present express, though it is possible that we are dealing with a mixture of families exhibiting the two forms of coupling.

From the other of the two F_3 plants chosen for further experiment (No.

6), we obtained a much more definite result. From it eight families, heterozygous in colour and pollen, were raised (Table II, Nos. 20-27). On

	Purple long	Purple round	Red long	Red round
No. 6 and its offspring, Nos. 20-27	583	26	24	170
Expectation on 15 : 1 : 1 : 15 basis	578	24	24	177

It is obvious that the numbers in this group of families accord very closely with the figures expected on a 15 : 1 : 1 : 15 basis; and the view that this is the system actually followed receives

adding together the results of these nine families, we obtain the following numbers:

confirmation from the distribution of the pollen and colour characters in the F₂ families from the Bush⁵ × Cupid crosses (Table I, p. 7), where the following figures were obtained:

	Purple long	Purple round	Red long	Red round
F ₂ families ex Bush × Cupid	131	6	5	42
Expectation on 15 : 1 : 1 : 15 basis	132·4	5·6	5·6	40·4

From the closeness with which the recorded results agree with expectation, it is evident that here again we are concerned with a 15 : 1 : 1 : 15 series. And when we come to deal with the inheritance of sterility in the Sweet Pea (p. 16), we shall meet with another case, in which we are undoubtedly concerned with a similar gametic series. We may take it, therefore, that there is good evidence for the existence of gametic coupling in the 15 : 1 : 1 : 15 series, as well as for the derivation of the families exhibiting the higher form from families in which the lower occurs. We have, then to recognize that in some families the coupling follows one system and in others another. Each process is, nevertheless, definite. As yet, however, we have not perceived any circumstance to which the distinction can be attributed.

In an earlier paragraph, we mentioned that, owing to the partial cou-

pling of long pollen with purple, we should expect hooded purples and hooded whites to be found very rarely with round pollen. When the gametes are produced in series of 16, i.e., 7 purple long, 1 purple round, 1 red long, and 7 red round, it is clear that the chances of two purple round gametes meeting are only 1 in 16², i.e., in 256 in families where reds occur. Of these 256 plants, 64 are homozygous for purple. Consequently, the chances of a homozygous purple having round pollen are 1 in 64. In families where hoods occur all the homozygous purples are hooded, and we should, therefore, look for one round hooded purple in 64. In the four F₁ families (Nos. 1-4), 1 round hooded purple occurred among 83 plants. Again, since the

⁵ The flower of the Bush plants used was a hooded white, exactly like that of Blanche Burpee.

hooded whites are homozygous for purple, on our view we should look for one with round pollen out of 64. Actually, one white hooded plant with round pollen occurred among the 18 F_2 families, where the hood was recorded (Nos. 1 and 4). Where expectation demands 1 in 64, experiment gave 2 hooded rounds in 101.

If, however, the coupling is on the 15 : 1 : 1 : 15 basis, the proportion of rounds among the hooded purples and whites should be only 1 in $32^2 \div 4$, i.e., 1 in 256. In No. 6 and its descendants, Nos. 20-27, only one of the 209 hooded purples had round pollen.

These complicated facts may be summarised thus: From an examination of the families where the hooded standard occurs, it is quite clear that in some of them the coupling of blue factor with long pollen is definitely distributed according to the system

15:1, and that in others it follows the system 7:1. There are also families which cannot confidently be referred to either class. Since the F_2 derived from Bl. Burpee by E.H. round followed the 7:1 system, the heterozygosis between erectness and hood cannot be regarded as the direct cause of the 15:1 distribution. The families showing that distribution came in F_3 and later generations from this cross, and the 15:1 system seems, therefore, to have been brought into operation by the omission of something which may be supposed to be carried on in those collateral families which follow the 7:1 system. Scrutiny of the various groups has, however, failed to discover any consistent difference between those of the 7:1 type and those of the 15:1 type. In the case of the Bush \times Cupid cross the 15:1 system appeared at once in F_2 .



Mendelian Proportions in a Mixed Population

G. H. HARDY

Reprinted by publisher's permission from *Science*, vol. 28, 1908, pp. 49-50.

This short paper has more of the air of a kindly old professor gently reprimanding an irrepressible student inclined to go off half-cocked than that of a major contribution to genetic thought. Hardy had noted the tendency of non-mathematically inclined biologists to make assumptions and draw conclusions based upon erroneous interpretations of the statistics in Mendel's work, and wrote this letter to the editor of Science to correct these errors. The consequences of the paper have been quite far-reaching, however, for it gave rise to the field of population genetics, which forms one of the primary

bases for the contribution of genetics to evolutionary thought. Hardy, as a mathematician, did not differentiate between the individual and the genes that individual carries, so he based his calculations of frequency on the numbers of homozygotes and heterozygotes in the population. Because of the redistribution of genes between individuals, his first generation, which was made up entirely of "pure" individuals, differs in proportions from his second generation, which includes heterozygotes. Geneticists soon recognized that the constancy and stability Hardy observed after his second generation existed equally in the transition from first to second, if one compares the total number of "A" and "a" genes in the population, rather than the numbers of different kinds of individuals. A direct consequence of this awareness is the "gene-pool" concept, which is concerned primarily with the total number of genes and their proportions in a population, and not with the appearance of the individuals carrying those genes. From the viewpoint that the number of genes in a gene pool tends to remain stable and unchanging comes the concept of evolution defined as any situation which tends to change the proportional distribution of genes in a gene pool. Hardy perceived several of the factors that could affect the proportional distribution, and pointed them out in his concluding paragraph. He missed one of the primary forces, however, in that the fruit of Darwin's thought, natural selection, is omitted.

The concept of stability of gene proportions in a population has come to be known as the "Hardy-Weinberg Law," as a consequence of another of those dramatic coincidences that were pointed out earlier, for Weinberg (*Über den Nachweis des Verebung beim Menschen*, 1908) pointed out the same facts at much the same time as did Hardy. This law still forms the core about which the field of population genetics revolves today.

TO THE EDITOR OF SCIENCE: I AM reluctant to intrude in a discussion concerning matters of which I have no expert knowledge, and I should have expected the very simple point which I wish to make to have been familiar to biologists. However, some remarks of Mr. Udny Yule, to which Mr. R. C. Punnett has called my attention, suggest that it may still be worth making.

In the *Proceedings of the Royal Society of Medicine* (Vol. I, p. 165) Mr. Yule is reported to have suggested, as a criticism of the Mendelian position, that if brachydactyly is dominant "in the course of time one would expect, in the absence of counteracting fac-

tors, to get three brachydactylous persons to one normal."

It is not difficult to prove, however, that such an expectation would be quite groundless. Suppose that Aa is a pair of Mendelian characters, A being dominant, and that in any given generation the numbers of pure dominants (AA), heterozygotes (Aa), and pure recessives (aa) are as $p:2q:r$. Finally, suppose that the numbers are fairly large, so that the mating may be regarded as random, that the sexes are evenly distributed among the three varieties, and that all are equally fertile. A little mathematics of the multiplication-table type is enough to show that

in the next generation the numbers will be as

$$(p+q)^2 : 2(p+q)(q+r) : (q+r)^2,$$

or as $p_1:2q_1:r_1$, say.

The interesting question is—in what circumstances will this distribution be the same as that in the generation before? It is easy to see that the condition for this is $q^2 = pr$. And since $q_1^2 = p_1r_1$, whatever the values of p , q and r may be, the distribution will in any case continue unchanged after the second generation.

Suppose, to take a definite instance, that A is brachydactyly, and that we start from a population of pure brachydactylous and pure normal persons, say in the ratio of 1:10,000. Then $p = 1$, $q = 0$, $r = 10,000$ and $p_1 = 1$, $q_1 = 10,000$, $r_1 = 100,000,000$. If brachydactyly is dominant, the proportion of brachydactylous persons in the second generation is 20,001:100,020,001, or practically 2:10,000, twice that in the first generation; and this proportion will afterwards have no tendency whatever to increase. If, on the other hand, brachydactyly were recessive, the proportion in the second generation would be 1:100,020,001, or practically 1:100,000,000, and this proportion would afterwards have no tendency to decrease.

In a word, there is not the slightest foundation for the idea that a dominant character should show a tendency to spread over a whole population, or that a recessive should tend to die out.

I ought perhaps to add a few words on the effect of the small deviations from the theoretical proportions

which will, of course, occur in every generation. Such a distribution as $p_1:2q_1:r_1$, which satisfies the condition $q_1^2 = p_1r_1$, we may call a *stable* distribution. In actual fact we shall obtain in the second generation not $p_1:2q_1:r_1$, but a slightly different distribution $p_1':2q_1':r_1'$, which is not "stable." This should, according to theory, give us in the third generation a "stable" distribution $p_2:2q_2:r_2$, also differing slightly from $p_1:2q_1:r_1$; and so on. The sense in which the distribution $p_1:2q_1:r_1$ is "stable" is this, that if we allow for the effect of casual deviations in any subsequent generation, we should, according to theory, obtain at the next generation a new "stable" distribution differing but slightly from the original distribution.

I have, of course, considered only the very simplest hypotheses possible. Hypotheses other than that of purely random mating will give different results, and, of course, if, as appears to be the case sometimes, the character is not independent of that of sex, or has an influence on fertility, the whole question may be greatly complicated. But such complications seem to be irrelevant to the simple issue raised by Mr. Yule's remarks.

P.S. I understand from Mr. Punnett that he has submitted the substance of what I have said above to Mr. Yule, and that the latter would accept it as a satisfactory answer to the difficulty that he raised. The "stability" of the particular ratio 1:2:1 is recognized by Professor Karl Pearson [*Phil. Trans. Roy. Soc. (A)*, vol. 203, p. 60].



Sex Limited Inheritance in *Drosophila*

T. H. MORGAN

Reprinted by publisher's permission from *Science*, vol. 32, 1910, pp. 120-122.

*Perhaps no other organism has contributed quite so much to man's knowledge of genetics as has the common fruit fly, *Drosophila melanogaster*. T. H. Morgan recognized the virtues of this animal as a subject of genetic experiments at an early date, and began to breed pure cultures shortly after the turn of the century. After a short period one of the most momentous occasions in the history of genetics took place. A white-eyed male appeared in a pure culture, and the study of genetic variation and its causes began. Morgan's paper discusses the experimental results of breeding tests using this male and his offspring.*

Again the specter of terminology causes difficulties. What Morgan refers to as "sex limited" is now known as sex linked. The term sex limited is now restricted to characteristics that occur only in a single sex. Morgan also discusses the occurrence of a "sex factor X" and, as in Bateson and Punnett, refers to the characteristic as being "X" or "no X." Other authors at much the same time had demonstrated that sex in the fruit fly was a consequence of unequal distribution of chromosomes, and Morgan actually demonstrates the presence of genes on the sex chromosome. At no time does he say this in his paper, however.

Two very important contributions are made in this paper. First is the discovery of a striking variant which breeds true in successive generations. Second is the possibility of assignment of a specific gene locus to a specific chromosome. An enormous and fertile field of investigation opened up as a result, as we shall see in subsequent papers.

IN A PEDIGREE CULTURE OF *DROSOPHILA* which had been running for nearly a year through a considerable number of generations, a male appeared with white eyes. The normal flies have brilliant red eyes.

The white-eyed male, bred to his

red-eyed sisters, produced 1,237 red-eyed offspring, (F_1), and 3 white-eyed males. The occurrence of these three white-eyed males (F_1) (due evidently to further sporting) will, in the present communication, be ignored.

The F_1 hybrids, inbred, produced:

2,459 red-eyed females,
1,011 red-eyed males,
782 white-eyed males.

No white-eyed females appeared. The new character showed itself therefore to be sex limited in the sense that it was transmitted only to the grandsons. But that the character is not incompatible with femaleness is shown by the following experiment.

The white-eyed male (mutant) was later crossed with some of his daughters (F_1), and produced:

129 red-eyed females,
132 red-eyed males,
88 white-eyed females,
86 white-eyed males.

The results show that the new character, white eyes, can be carried over to the females by a suitable cross, and is in consequence in this sense not limited to one sex. It will be noted that the four classes of individuals occur in approximately equal numbers (25 per cent.).

An Hypothesis to Account for the Results.—The results just described can be accounted for by the following hypothesis. Assume that all of the spermatozoa of the white-eyed male carry the "factor" for white eyes "W"; that half of the spermatozoa carry a sex factor "X," the other half lack it, *i. e.*, the male is heterozygous for sex. Thus the symbol for the male is "WWX," and for his two kinds of spermatozoa $WX-W$.

Assume that all of the eggs of the red-eyed female carry the red-eyed "factor" R; and that all of the eggs (after reduction) carry one X each, the symbol for the red-eyed female will be therefore RRXX and that for her eggs will be RX-RX.

When the white-eyed male (sport) is crossed with his red-eyed sisters, the following combinations result:

WX - W (male)
RX - RX (female)
$\overline{RWXX \text{ (50\%)} - RWX \text{ (50\%)}}$
Red female Red male

When these F_1 individuals are mated, the following table shows the expected combinations that result:

RX - WX (F_1 female)
RX - W (F_1 male)
$\overline{RRXX - RWXX - RWX - WWX}$
(25%) (25%) (25%) (25%)
Red Red Red White
female female male male

It will be seen from the last formulæ that the outcome is Mendelian in the sense that there are three reds to one white. But it is also apparent that all of the whites are confined to the male sex.

It will also be noted that there are two classes of red females—one pure RRXX and one hybrid RWXX—but only one class of red males (RWX). This point will be taken up later. In order to obtain these results it is necessary to assume, as in the last scheme, that, when the two classes of the spermatozoa are formed in the F_1 red male (RWX), R and X go together—otherwise the results will not follow (with the symbolism here used). This all-important point can not be fully discussed in this communication.

The hypothesis just utilized to explain these results first obtained can be tested in several ways.

VERIFICATION OF HYPOTHESIS

First Verification.—If the symbol for the white male is WWX, and for the white female WWXX, the germ cells will be $WX-W$ (male) and $WX-WX$ (female), respectively. Mated, these individuals should give

WX - W (male)
WX - WX(female)
$\overline{WWXX \text{ (50\%)} - WWX \text{ (50\%)}}$
White female White male

All of the offspring should be white, and male and female in equal numbers; this in fact is the case.

Second Verification. — As stated, there should be two classes of females in the F_2 generation, namely, RRXX and RWXX. This can be tested by pairing individual females with white males. In the one instance (RRXX) all the offspring should be red—

$$\begin{array}{c} \text{RX} - \text{RX} \text{ (female)} \\ \text{WX} - \text{W} \text{ (male)} \\ \hline \text{RWXX} - \text{RWX} \end{array}$$

and in the other instance (RWXX) there should be four classes of individuals in equal numbers, thus:

$$\begin{array}{c} \text{RX} - \text{WX} \text{ (female)} \\ \text{WX} - \text{W} \text{ (male)} \\ \hline \text{RWXX} - \text{WWXX} - \text{RWX} - \text{WWX} \end{array}$$

Tests of the F_2 red females show in fact that these two classes exist.

Third Verification. — The red F_1 females should all be RWXX, and should give with any white male the four combinations last described. Such in fact is found to be the case.

Fourth Verification. — The red F_1 males (RWX) should also be heterozygous. Crossed with white females (WWXX) all the female offspring should be red-eyed, and all the male offspring white-eyed, thus:

$$\begin{array}{c} \text{RX} - \text{W} \text{ (red male)} \\ \text{WX} - \text{WX} \text{ (white female)} \\ \hline \text{RWXX} - \text{WWX} \end{array}$$

Here again the anticipation was verified, for all of the females were red-eyed and all of the males were white-eyed.

CROSSING THE NEW TYPE WITH WILD MALES AND FEMALES

A most surprising fact appeared when a white-eyed female was paired

to a wild, red-eyed male, *i. e.*, to an individual of an unrelated stock. The anticipation was that wild males and females alike carry the factor for red eyes, but the experiments showed that all wild males are heterozygous for red eyes, and that all the wild females are homozygous. Thus when the white-eyed female is crossed with a wild red-eyed male, all of the female offspring are red-eyed, and all of the male offspring white-eyed. The results can be accounted for on the assumption that the wild male is RWX. Thus:

$$\begin{array}{c} \text{RX} - \text{W} \text{ (red male)} \\ \text{WX} - \text{WX} \text{ (white female)} \\ \hline \text{RWXX (50\%)} - \text{WWX (50\%)} \end{array}$$

The converse cross between a white-eyed male RWX and a wild, red-eyed female shows that the wild female is homozygous both for X and for red eyes. Thus:

$$\begin{array}{c} \text{WX} - \text{W} \text{ (white male)} \\ \text{RX} - \text{RX} \text{ (red female)} \\ \hline \text{RWXX (50\%)} - \text{RWX (50\%)} \end{array}$$

The results give, in fact, only red males and females in equal numbers.

GENERAL CONCLUSIONS

The most important consideration from these results is that in every point they furnish the converse evidence from that given by *Abraxas* as worked out by Punnett and Raynor. The two cases supplement each other in every way, and it is significant to note in this connection that in nature only females of the sport *Abraxas lacticolor* occur, while in *Drosophila* I have obtained only the male sport. Significant, too, is the fact that analysis of the result shows that the wild female *Abraxas grossulariata* is heterozygous for color and sex, while in *Drosophila* it is the male that is heterozygous for these two characters.

Since the wild males (RWX) are heterozygous for red eyes, and the female (RXRX) homozygous, it seems probable that the sport arose from a change in a single egg of such a sort that instead of being RX (after reduction) the red factor dropped out, so that RX became WX or simply OX. If this view is correct it follows that the mutation took place in the egg of a female from which a male was produced by combination with the sperm carrying no X, no R (or W in our formulæ). In other words, if the formula for the eggs of the normal female is RX-RX, then the formula for the particular egg that sported will be WX; *i. e.*, one R dropped out of the egg leaving it WX (or no R and one X), which may be written OX. This egg we assume was fertilized by a male-producing sperm. The formula for the two classes of spermatozoa is RX-O. The latter, O, is the male-producing sperm, which combining with the egg OX (see above) gives OOX (or WWX), which is the formula for the white-eyed male mutant.

The transfer of the new character (white eyes) to the female (by crossing a white-eyed male, OOX to a

heterozygous female (F_1)) can therefore be expressed as follows:

OX — O (white male)
<u>RX — OX (F_1 female)</u>
R
X
Red
female
male
O
W
White
female
male
O
W
White
male

It now becomes evident why we found it necessary to assume a coupling of R and X in one of the spermatozoa of the red-eyed F_1 hybrid (RXO). The fact is that this R and X are combined, and have never existed apart.

It has been assumed that the white-eyed mutant arose by a male-producing sperm (O) fertilizing an egg (OX) that had mutated. It may be asked what would have been the result if a female-producing sperm (RX) had fertilized this egg (OX)? Evidently a heterozygous female RXOX would arise, which, fertilized later by any normal male (RX-O) would produce in the next generation pure red females RRXX, red heterozygous females RXOX, red males RXO, and white males OOX (25 per cent.). As yet I have found no evidence that white-eyed sports occur in such numbers. Selective fertilization may be involved in the answer to this question.



The Linear Arrangement of Six Sex-Linked Factors in *Drosophila*, as Shown by Their Mode of Association

A. H. STURTEVANT

Reprinted by author's and publisher's permission from *Journal of Experimental Zoology*, vol. 14, 1913, pp. 43-59.

Sturtevant's paper takes advantage of the previous researches of many different people, adds to them a series of detailed experiments, and results in definite proof that the factors (genes) are arranged in a linear sequence along the chromosome. Actually, Sturtevant regarded his work as a substantiation of the hypothesis that the genes were carried by the chromosomes, primarily as a consequence of the linear nature of their arrangement, for the only possible method of linear arrangement within a cell would be along the length of the chromosome.

*The work provided the basis for the construction of chromosome maps in many species besides *Drosophila*, because the method used is applicable in any species in which linkage groups have been discovered. It should be kept in mind that these are proportional locations, and therefore show only relative, not actual, positions.*

Sturtevant's paper is brief, concise, and to the point. It has the added virtue of careful presentation of the problems created as a consequence of the research, and the analysis of possible objections to the results. Careful study of this paper will greatly repay anyone interested in the technique of presentation of research results.

HISTORICAL

THE PARALLEL BETWEEN THE BEHAVIOR of the chromosomes in reduction and that of Mendelian factors in segregation was first pointed out by Sutton ('02) though earlier in the same year Boveri ('02) had referred to a possible connection (*loc. cit.*, footnote 1, p.

81). In this paper and others Boveri brought forward considerable evidence from the field of experimental embryology indicating that the chromosomes play an important rôle in development and inheritance. The first attempt at connecting any given somatic character with a definite chromosome came with McClung's ('02)

suggestion that the accessory chromosome is a sex-determiner. Stevens ('05) and Wilson ('05) verified this by showing that in numerous forms there is a sex chromosome, present in all the eggs and in the female-producing sperm, but absent, or represented by a smaller homologue, in the male-producing sperm. A further step was made when Morgan ('10) showed that the factor for color in the eyes of the fly *Drosophila ampelophila* follows the distribution of the sex-chromosome already found in the same species by Stevens ('08). Later, on the appearance of a sex-linked wing mutation in *Drosophila*, Morgan ('10 a, '11) was able to make clear a new point. By crossing white eyed, long winged flies to those with red eyes and rudimentary wings (the new sex-linked character) he obtained, in F_2 , white eyed rudimentary winged flies. This could happen only if 'crossing over' is possible; which means, on the assumption that both of these factors are in the sex-chromosomes, that an interchange of materials between homologous chromosomes occurs (in the female only, since the male has only one sex-chromosome). A point not noticed at this time came out later in connection with other sex-linked factors in *Drosophila* (Morgan '11 d). It became evident that some of the sex-linked factors are associated, i.e., that crossing over does not occur freely between some factors, as shown by the fact that the combinations present in the F_1 flies are much more frequent in F_2 than are new combinations of the same characters. This means, on the chromosome view, that the chromosomes, or at least certain segments of them, are more likely to remain intact during reduction than they are to interchange materials.¹ On the basis

of these facts Morgan ('11 c, '11 d) has made a suggestion as to the physical basis of coupling. He uses Janssens' ('09) chiasmatype hypothesis as a mechanism. As he expresses it (Morgan '11 c):

If the materials that represent these factors are contained in the chromosomes, and if those that "couple" be near together in a linear series, then when the parental pairs (in the heterozygote) conjugate like regions will stand opposed. There is good evidence to support the view that during the strepsinema stage homologous chromosomes twist around each other, but when the chromosomes separate (split) the split is in a single plane, as maintained by Janssens. In consequence, the original materials will, for short distances, be more likely to fall on the same side of the split, while remoter regions will be as likely to fall on the same side as the last, as on the opposite side. In consequence, we find coupling in certain characters, and little or no evidence at all of coupling in other characters, the difference depending on the linear distance apart of the chromosomal materials that represent the factors. Such an explanation will account for all the many phenomena that I have observed and will explain equally, I think, the other cases so far described. The results are a simple mechanical result of the location of the materials in the chromosomes, and of the method of union of homologous chromosomes, and the proportions that result are not so much the expression of a numerical system as of the relative location of the factors in the chromosomes.

SCOPE OF THIS INVESTIGATION

It would seem, if this hypothesis be correct, that the proportion of 'crossovers' could be used as an index of the distance between any two factors. Then by determining the distances (in the above sense) between A and B and between B and C, one should be able to predict AC. For, if proportion of

¹ It is interesting to read, in this connection, Lock's ('06, p. 248-253) discussion of the matter.

cross-overs really represents distance, AC must be approximately, either AB plus BC, or AB minus BC, and not any intermediate value. From purely mathematical considerations, however, the sum and the difference of the proportion of cross-overs between A and B and those between B and C are only *limiting* values for the proportion of cross-overs between A and C. By using several pairs of factors one should be able to apply this test in several cases. Furthermore, experiments involving three or more sex-linked allelomorphic pairs together should furnish another and perhaps more crucial test of the view. The present paper is a preliminary report of the investigation of these matters.

I wish to thank Dr. Morgan for his kindness in furnishing me with material for this investigation, and for his encouragement and the suggestions he has offered during the progress of the work. I have also been greatly helped by numerous discussions of the theoretical side of the matter with Messrs. H. J. Muller, E. Altenburg, C. B. Bridges, and others. Mr. Muller's suggestions have been especially helpful during the actual preparation of the paper.

THE SIX FACTORS CONCERNED

In this paper I shall treat of six sex-linked factors and their inter-relationships. These factors I shall discuss in the order in which they seem to be arranged.

B stands for the black factor. Flies recessive with respect to it (b) have yellow body color. The factor was first described and its inheritance given by Morgan ('11 a).

C is a factor which allows color to appear in the eyes. The white eyed fly (first described by Morgan '10) is now known to be always recessive with respect both to C and to the next factor.

O. Flies recessive with respect to O(o) have eosin eyes. The relation between C and O has been explained by Morgan in a paper now in print and about to appear in the Proceedings of the Academy of Natural Sciences in Philadelphia.

P. Flies with p have vermillion eyes instead of the ordinary red (Morgan '11 d).

R. This and the next factor both affect the wings. The normal wing is RM. The rM wing is known as miniature, the Rm as rudimentary, and the rm as rudimentary-miniature. This factor R is the one designated L by Morgan ('11 d) and Morgan and Cattell ('12). The L of Morgan's earlier paper ('11) was the next factor.

M. This has been discussed above, under R. The miniature and rudimentary wings are described by Morgan ('11 a).

The relative position of these factors is B, $\frac{C}{O}$, P, R, M. C and O are placed at the same point because they are completely linked. Thousands of flies had been raised from the cross CO (red) by co (white) before it was known that there were two factors concerned. The discovery was finally made because of a mutation and not through any crossing over. It is obvious, then, that unless coupling strength be variable, the same gametic ratio must be obtained whether, in connection with other allelomorphic pairs, one uses CO (red) as against co (white), Co (eosin) against co (white), or CO (red) against Co (eosin) (the cO combination is not known).

METHOD OF CALCULATING STRENGTH OF ASSOCIATION

In order to illustrate the method used for calculating the gametic ratio I shall use the factors P and M. The cross used in this case was, long

winged, vermillion-eyed female by rudimentary winged, red-eyed male. The analysis and results are seen in Table 1.

It is of course obvious from the figures that there is something peculiar about the rudimentary winged flies, since they appear in far too small numbers. This point need not detain us here, as it always comes up in connection with rudimentary crosses, and is

TABLE 1

	Long vermillion	♀—MpX MpX
	Rudimentary red	♂—mPX
F_1	MpX mPX—long red	♀
	MpX —long vermillion	♂
Gametes F_1		
Eggs	—MPX mPX MpX mpX	
Sperm—MpX		
F_2		
MPX MpX } mPX MpX }	—long red ♀—451	
MpX MpX } mpX MpX }	—long vermillion ♀—417	
MPX	—long red ♂—105	
mPX	—rudimentary red ♂—33	
MpX	—long vermillion ♂—316	
mpX	—rudimentary vermillion ♂—4	

being investigated by Morgan. The point of interest at present is the linkage. In the F_2 generation the original combinations, red rudimentary and vermillion long, are much more frequent in the males (allowing for the low viability of rudimentary) than are the two new or cross-over combinations, red long and vermillion rudimentary. It is obvious from the analysis that no evidence of association can be found in the females, since the M present in all female-producing sperm masks m when it occurs. But the ratio of cross-overs in the gametes is given

without complication by the F_2 males, since the male-producing sperm of the F_1 male bore no sex-linked genes. There are in this case 349 males in the non-cross-over classes and 109 in the cross-overs. The method which has seemed most satisfactory for expressing the relative position of factors, on the theory proposed in the beginning of this paper, is as follows. The unit of 'distance' is taken as a portion of the chromosome of such length that, on the average, one cross-over will occur in it out of every 100 gametes formed. That is, per cent of cross-overs, is used as an index of distance. In the case of P and M there occurred 109 cross-overs in 405 gametes, a ratio of 26.9 in 100; 26.9, the per cent of cross-overs, is considered as the 'distance' between P and M.

THE LINEAR ARRANGEMENT OF THE FACTORS

Table 2 shows the proportion of cross-overs in those cases which have been worked out. The detailed results of the crosses involved are given at the end of this paper. The 16287 cases for B and CO are from Dexter ('12). Inasmuch as C and O are completely linked I have added the numbers for C, for O, and for C and O taken together, giving the total results in the lines beginning (C, O) P, B (C, O), etc., and have used these figures, instead of the individual C, O, or CO results, in my calculations. The fractions in the column marked 'proportion of cross-overs' represent the number of cross-overs (numerator) to total available gametes (denominator).

As will be explained later, one is more likely to obtain accurate figures for distances if those distances are short, i.e., if the association is strong.

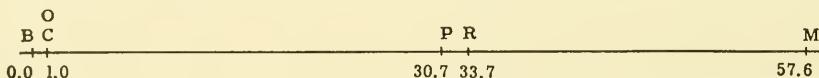


TABLE 2

Factors Concerned	Proportion of Cross-overs	Per Cent of Cross-overs
BCO	193 16287	1.2
BO	2 373	0.5
BP	1464 4551	32.2
BR	115 324	35.5
BM	260 693	37.6
COP	224 748	30.0
COR	1643 4749	34.6
COM	76 161	47.2
OP	247 836	29.4
OR	183 538	34.0
OM	218 404	54.0
CR	236 829	28.5
CM	112 333	33.6
B(C, O)	214 21736	1.0
(C, O)P	471 1584	29.7
(C, O)R	2062 6116	33.7
(C, O)M	406 898	45.2
PR	17 573	3.0
PM	109 405	26.9

For this reason I shall, in so far as possible, use the per cent of cross-overs between adjacent points in mapping out the distances between the various

factors. Thus, B (C, O), (C, O) P, PR, and PM form the basis of the diagram. The figures on the diagram represent calculated distances from B.

Of course there is no knowing whether or not these distances as drawn represent the actual relative spacial distances apart of the factors. Thus the distance CP may in reality be shorter than the distance BC, but what we do know is that a break is far more likely to come between C and P than between B and C. Hence, either CP is a long space, or else it is for some reason a weak one. The point I wish to make here is that we have no means of knowing that the chromosomes are of uniform strength, and if there are strong or weak places, then that will prevent our diagram from representing actual relative distances—but, I think, will not detract from its value as a diagram.

Just how far our theory stands the test is shown by Table 3, giving observed per cent of cross-overs, and distances as calculated from the figures given in the diagram of the chromosome. Table 3 includes all pairs of factors given in Table 2 but not used in the preparation of the diagram.

It will be noticed at once that the long distances, BM, and (C, O)M, give smaller per cent of cross-overs, than the calculation calls for. This is a point which was to be expected, and will be discussed later. For the present we may dismiss it with the statement that it is probably due to the occurrence of two breaks in the same chromosome, or 'double crossing over.' But in the case of the shorter distances the correspondence with expectation is perhaps as close as was to be expected with the small numbers that are available. Thus, BP is 3.2 less than BR, the difference expected being 3.0. (C, O)R is less than

TABLE 3

Factors	Calculated Distance	Observed Per Cent of Cross-overs
BP	30.7	32.2
BR	33.7	35.5
BM	57.6	37.6
(C, O)R	32.7	33.7
(C, O)M	56.6	45.2

BR by 1.8 instead of by 1.0. It has actually been found possible to predict the strength of association between two factors by this method, fair approximations having been given for BR and for certain combinations involving factors not treated in this paper, before the crosses were made.

DOUBLE CROSSING OVER

On the chiasmatype hypothesis it will sometimes happen, as shown by Dexter ('12) and intimated by Morgan ('11 d) that a section of, say, maternal chromosome will come to have paternal elements at both ends, and perhaps more maternal segments beyond these. Now if this can happen it introduces a complication into the results. Thus, if a break occurs between B and P, and another between P and M, then, unless we can follow P also, there will be no evidence of crossing over between B and M, and the fly hatched from the resulting gamete will be placed in the non-cross-over class, though in reality he represents two cross-overs. In order to see if double crossing over really does occur it is necessary to use three or more sex-linked allelomorphic pairs in the same experiment. Such cases have been reported by Morgan ('11 d) and Morgan and Cattell ('12) for the factors B, CO, and R. They made such crosses as long gray red by miniature yellow white, and long yellow red by miniature gray white, etc. The details

and analyses are given in the original papers, and for our present purpose it is only the flies that are available for observations on double crossing over that are of interest. Table 4 gives a graphical representation of what happened in the 10495 cases.

Double crossing over does then occur, but it is to be noted that the occurrence of the break between B and CO tends to prevent that between CO and R (or vice versa). Thus where B and CO did not separate, the gametic ratio for CO and R was about 1 to 2, but in the cases where B and CO did separate it was about 1 to 6.5.

Three similar cases from my own results, though done on a smaller scale, are given in the table at the end of this paper. The results are represented in Tables 5, 6 and 7.

It will be noted that here also the evidence, so far as it goes, indicated that the occurrence of one cross-over makes another one less likely to occur in the same gamete. In the case of BOPR there was an opportunity for triple crossing over, but it did not occur. Of course, on the view here presented there is no reason why it should not occur, if enough flies were raised. An examination of the figures will show that it was not to be expected in such small numbers as are here given. So far as I know there is, at present, no evidence that triple crossing over takes place, but it seems highly probable that it will be shown to occur.²

Unfortunately, in none of the four cases given above are two comparatively long distances involved, and in only one are there enough figures to form a fair basis for calculation, so that it seems as yet hardly possible to determine how much effect double

² A case of triple crossing over within the distance CR was observed after this paper went to press.

TABLE 4

NO CROSSING OVER	SINGLE CROSSING OVER	DOUBLE CROSSING OVER	
B CO R 6972	B CO R 3454	B CO R 60	B CO R 9

TABLE 5

NO CROSSING	SINGLE CROSSING OVER	DOUBLE CROSSING OVER	
O P R 194	O P R 102	O P R 11	O P R 1

TABLE 6

NO CROSSING	SINGLE CROSSING OVER	DOUBLE CROSSING OVER	
B O M 278	B O M 160	B O M 1	B O M 0

TABLE 7

B O P R 393	B O P R 203	B O P R 19	B O P R 6	B O P R 2	B O P R 1	B O P R 1	B O P R 0
-------------------------	-------------------------	------------------------	-----------------------	-----------------------	-----------------------	-----------------------	-----------------------

crossing over has in pulling down the observed per cent of cross-overs in the case of BM and (C, O)M. Whether or not this effect is partly counter-bal-

anced by triple crossing over must also remain unsettled as yet. Work now under way should furnish answers to both these questions.

TABLE 8

(The meaning of the phrase 'proportion of cross-overs' is given on p. 68)

BO. P_1 : gray eosin ♀ × yellow red ♂
 F_1 : gray red ♀ × gray eosin ♂
 F_2 : ♀ ♀, g.r. 241, g.e. 196
 ♂ ♂, g.r. 0, g.e. 176, y.r. 195, y.e. 2

Proportion of cross-overs, $\frac{2}{373}$

BP. P_1 : gray red ♀ × yellow vermillion ♂
 F_1 : gray red ♀ × gray red ♂

TABLE 8—Continued.

- F₂: ♀ ♀, g.r. 98;
 ♂ ♂, g.r. 59, g.v. 16, y.r. 24, y.v. 33
 Back cross, F₁ gray red ♀ ♀ from above × yellow vermilion ♂ ♂
- F₂: ♀ ♀, g.r. 31, g.v. 11, y.r. 12, y.v. 41
 ♂ ♂, g.r. 23, g.v. 13, y.r. 8, y.v. 21
 P₁: gray vermilion ♀ × yellow red ♂
- F₁: gray red ♀ × gray vermilion ♂
- F₂: ♀ ♀, g.r. 199, g.v. 182
 ♂ ♂, g.r. 54, g.v. 149, y.r. 119, y.v. 41
 P₁: yellow vermilion ♀ × gray red ♂
- F₁: gray red ♀ × yellow vermilion ♂
- F₂: ♀ ♀, g.r. 472, g.v. 240, y.r. 213, y.v. 414
 ♂ ♂, g.r. 385, g.v. 186, y.r. 189, y.v. 324
 F₁: gray vermilion × yellow red (sexes not recorded)
- F₁: gray red ♀ ♀. These were mated to yellow vermilion ♂ ♂ of other stock
- F₂: ♀ ♀, g.r. 50, g.v. 96, y.r. 68, y.v. 41
 ♂ ♂, g.r. 44, g.v. 105, y.r. 86, y.v. 47

Proportion of cross-overs, adding ♀ ♀ from BOPR (below), $\frac{1464}{4551}$

- BR. P₁: miniature yellow ♀ × long gray ♂
 F₁: long gray ♀ × miniature yellow ♂
- F₂: ♀ ♀, l.g. 14, l.y. 2, m.g. 7, m.y. 6
 ♂ ♂, l.g. 10, l.y. 1, m.g. 6, m.y. 8
 P₁: long yellow ♀ × miniature gray ♂
 F₁: long gray ♀ × long yellow ♂
- F₂: ♀ ♀, l.g. 148, l.y. 130
 ♂ ♂, l.g. 51, l.y. 82, m.g. 89, m.y. 48

Proportion of cross-overs, $\frac{115}{324}$

- BM. P₁: long yellow ♀ × rudimentary gray ♂
 F₁: long gray ♀ × long yellow ♂
- F₁: ♀ ♀, l.g. 591, l.y. 549
 ♂ ♂, l.g. 228, l.y. 371, r.g. 20, r.y. 3
 P₁: long gray ♀ × rudimentary yellow ♂
 F₁: long gray ♀ × long gray ♂
- F₂: ♀ ♀, l.g. 152
 ♂ ♂, l.g. 42, l.y. 29, r.g. 0, r.y. 0

Proportion of cross-overs, $\frac{260}{693}$

- COP. P₁: vermilion ♀ × white ♂
 F₁: red ♀ × vermilion ♂
- F₂: ♀ ♀, r. 320, v. 294
 ♂ ♂, r. 86, v. 206, w. 211
 (7 of the vermilion ♀ ♀ known from tests to be CC, 2 known to be Cc. 7 white ♂ ♂ Pp, 2 pp.)
 Back cross, F₁ red ♀ ♀ from above × white ♂ ♂, gave
- F₂: ♀ ♀, r. 195, w. 227,
 ♂ ♂, r. 66, v. 164, w. 184
 Out cross, F₁ ♀ ♀ as above × white ♂ ♂ recessive in P, gave
- F₂: ♀ ♀, r. 35, v. 65, w. 98
 ♂ ♂, r. 33, v. 75, w. 95

TABLE 8—Continued.

Proportion of cross-overs, $\frac{224}{748}$

COR. P₁: miniature white ♀ × long red ♂
 F₁: long red ♀ × miniature white ♂
 F₂: ♀ ♀, l.r. 193, l.w. 109, m.r. 124, m.w. 208
 ♂ ♂, l.r. 202, l.w. 114, m.r. 123, m.w. 174
 P₁: long white ♀ × miniature red ♂
 F₁: long red ♀ × long white ♂
 F₂: ♀ ♀, l.r. 194, l.w. 160
 ♂ ♂, l.r. 52, l.w. 124, m.r. 97, m.w. 41

Proportion of cross-overs, $\frac{563}{1561}$; or, adding such available figures from

Morgan ('11 d) and Morgan and Cattell ('12) as are not complicated
 by the presence of yellow or brown flies, $\frac{1643}{4749}$

COM. P₁: long white ♀ × rudimentary red ♂
 F₁: long red ♀ × long white ♂
 F₂: ♀ ♀, l.r. 157, l.w. 127
 ♂ ♂, l.r. 74, l.w. 82, ru.r. 3, ru.w. 2

Proportion of cross-overs, $\frac{76}{161}$

OP. P₁: black red ♀ × black eosin-vermilion ♂
 F₁: black red ♀ × black red ♂
 F₂: (all black), ♀ ♀, r. 885
 ♂ ♂, r. 321, v. 125, e. 122, e.-v. 268

Proportion of cross-overs, $\frac{247}{836}$

OR. P₁: long red ♀ × miniature eosin ♂
 F₁: long red ♀ × long red ♂
 F₂: ♀ ♀, l.r. 408
 ♂ ♂, l.r. 145, l.e. 67, m.r. 70, m.e. 100
 P₁: long eosin ♀ × miniature red ♂
 F₁: long red ♀ × long eosin ♂
 F₂: ♀ ♀, l.r. 100, l.e. 95
 ♂ ♂, l.r. 27, l.e. 54, m.r. 56, m.e. 19

Proportion of cross-overs, $\frac{183}{538}$

OM. P₁: long eosin ♀ × rudimentary red ♂
 F₁: long red ♀ × long eosin ♂
 F₂: ♀ ♀, l.r. 368, l.e. 266
 ♂ ♂, l.r. 194, l.e. 146, ru.r. 40, ru.e. 24

Proportion of cross-overs, $\frac{218}{404}$

CR. P₁: long white ♀ × miniature eosin ♂
 F₁: long eosin ♀ × long white ♂

TABLE 8—Continued.

- F₂: ♀ ♀, l.e. 185, l.w. 205
 ♂ ♂, l.e. 54, l.w. 147, m.e. 149, m.w. 42
 P₁: long eosin ♀ × miniature white ♂
 F₁: long eosin ♀ × long eosin ♂
 F₂: ♀ ♀, l.e. 527
 ♂ ♂, l.e. 169, l.w. 85, m.e. 55, m.w. 128

Proportion of cross-overs, $\frac{236}{829}$

- CM. P₁: long white ♀ × rudimentary eosin ♂
 F₁: long eosin ♀ × long white ♂
 F₂: ♀ ♀, l.e. 328, l.w. 371
 ♂ ♂, l.e. 112, l.w. 217, ru.e. 4, ru.w. 0

Proportion of cross-overs, $\frac{112}{333}$

- PR. P₁: long vermillion (yellow) ♀ × miniature red (yellow) ♂
 F₁: long red yellow ♀ × long vermillion yellow ♂
 F₂: (all y.) ♀ ♀, l.r. 138, l.v. 110
 ♂ ♂, l.r. 8, l.v. 117, m.r. 97, m.v. 1
 P₁: long vermillion (gray) ♀ × miniature red ♂
 F₁: long red ♀ × long vermillion ♂
 F₂: ♀ ♀, l.r. 116, l.v. 110
 ♂ ♂, l.r. 2, l.v. 81, m.r. 96, m.v. 1
 P₁: miniature red ♀ × long vermillion ♂
 F₁: long red ♀ × miniature red ♂
 F₁: ♀ ♀, l.r. 45, m.r. 49
 ♂ ♂, l.r. 1, l.v. 27, m.r. 26, m.v. 0
 F₁ long red ♀ ♀ from above × miniature red ♂ ♂ of other stock, gave
 F₂: ♀ ♀, l.r. 74, m.r. 52
 ♂ ♂, l.r. 3, l.v. 66, m.r. 46, m.v. 1

Proportion of cross-overs, $\frac{17}{573}$

- PM. P₁: long vermillion ♀ × rudimentary red ♂
 F₁: long red ♀ × long vermillion ♂
 F₂: ♀ ♀, l.r. 451, l.v. 417
 ♂ ♂, l.r. 105, l.v. 316, ru.r. 33, ru.v. 4

Proportion of cross-overs, $\frac{109}{405}$

- OPR. P₁: long vermillion ♀ × miniature eosin ♂
 F₁: long red ♀ × long vermillion ♂
 F₂: ♀ ♀, l.r. 205, l.v. 182
 ♂ ♂, l.r. 1, l.v. 109, l.e. 8, l.e.-v. 53, m.r. 49, m.v. 3, m.e. 85, m.e.-v. 0

- BOM. P₁: long red yellow ♀ × rudimentary eosin gray ♂
 F₁: long red gray ♀ × long red yellow ♂
 F₂: ♀ ♀, l.r.g. 530, l.r.y. 453
 ♂ ♂, l.r.g. 1, l.r.y. 274, l.e.g. 156, l.e.y. 0, ru.r.g. 0, ru.r.y. 4,
 ru.e.g. 4, ru.c.y. 0

- BOPR. P₁: long vermillion brown ♀ × miniature eosin black ♂
 F₁: long red black ♀ × long vermillion brown ♂
 F₂: ♀ ♀, l.r.bl. 305, l.r.br. 113, l.v.bl. 162, lv.br. 256
 ♂ ♂, l.r.bl. 0, l.r.br. 2, l.v.bl. 3, l.v.br. 185, l.e.bl. 9, l.e.br. 0,
 l.e.-v.bl. 127, l.e.-v.br. 0, m.r.bl. 1, m.r.br. 76, m.v.bl. 1, m.v.br. 10,
 m.e.bl. 208, m.e.br. 3, m.e.-v.bl. 0, m.e.-v.br. 0

POSSIBLE OBJECTIONS
TO THESE RESULTS

It will be noted that there appears to be some variation in coupling strength. Thus, I found (CO)R to be 36.7; Morgan and Cattell obtained the result 33.9; for OR I got 34.0, and for CR, 28.5. The standard error for the difference between (CO)R (all figures) and CR is 1.84 per cent, which means that a difference of 5.5 per cent is probably significant (Yule '11, p. 264). The observed difference is 6.1 per cent, showing that there is some complication present. Similarly, BM gave 37.6, while OM gave 54.0—and BOM gave 36.7 for BM, and 36.5 for OM. There is obviously some complication in these cases, but I am inclined to think that the disturbing factor discussed below (viability) will explain this. However, experiments are now under way to test the effect of certain external conditions on coupling strength. It will be seen that on the whole when large numbers are obtained in different experiments and are averaged, a fairly consistent scheme results. Final judgment on this matter must, however, be withheld until the subject can be followed up by further experiments.

Another point which should be considered in this connection is the effect of differences in viability. In the case of P and M, used above as an illustration, the rudimentary winged flies are much less likely to develop than are the longs. Now if the viability of red and vermillion is different, then the longs do not give a fair measure of the linkage, and the rudimentaries, being present in such small numbers, do not even up the matter. It is probable that there is no serious error due to this cause except in the case of rudimentary crosses, since the two sides will tend to even up, unless one is very

much less viable than the other, and this is true only in the case of rudimentary. It is worth noting that the only serious disagreements between observation and calculation occur in the case of rudimentary crosses (BM, and (CO)M). Certain data of Morgan's now in print, and further work already planned, will probably throw considerable light on the question of the position and behavior of this factor M.

SUMMARY

It has been found possible to arrange six sex-linked factors in *Drosophila* in a linear series, using the number of cross-overs per 100 cases as an index of the distance between any two factors. This scheme gives consistent results, in the main.

A source of error in predicting the strength of association between untried factors is found in double crossing over. The occurrence of this phenomenon is demonstrated, and it is shown not to occur as often as would be expected from a purely mathematical point of view, but the conditions governing its frequency are as yet not worked out.

These results are explained on the basis of Morgan's application of Janssens' chiasmatype hypothesis to associative inheritance. They form a new argument in favor of the chromosome view of inheritance, since they strongly indicate that the factors investigated are arranged in a linear series, at least mathematically.

LITERATURE CITED

- Boveri, T., 1902 "Ueber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns." *Verb, Phys.-Med. Ges Würzburg*, N.F., Bd. 35, p. 67.
 Dexter, J. S., 1912 On coupling of certain sex-linked characters in *Drosophila*. *Biol. Bull.*, vol. 23, p. 183.

- Janssens, F. A., 1909 La théorie de la chiasmatypie. *La Cellule*, tom. 25, p. 389.
- Lock, R. H., 1906 Recent progress in the study of variation, heredity, and evolution. London and New York.
- McClung, C. E., 1902 The accessory chromosome—sex determinant? *Biol. Bull.*, vol. 3, p. 43.
- Morgan, T. H., 1910 Sex-limited inheritance in *Drosophila*. *Science*, n.s., vol. 32, p. 1.
- 1910 a The method of inheritance of two sex-limited characters in the same animal. *Proc. Soc. Exp. Biol. Med.*, vol. 8, p. 17.
- 1911 The application of the conception of pure lines to sex-limited inheritance and to sexual dimorphism. *Amer. Nat.*, vol. 45, p. 65.
- 1911 a The origin of nine wing mutations in *Drosophila*. *Science*, n.s., vol. 33, p. 496.
- 1911 b The origin of five mutations in eye color in *Drosophila* and their modes of inheritance. *Science*, n.s., vol. 33, p. 534.
- 1911 c Random segregation versus coupling in Mendelian inheritance. *Science*, n.s., vol. 34, p. 384.
- 1911 d An attempt to analyze the constitution of the chromosomes on the basis of sex-limited inheritance in *Drosophila*. *Jour. Exp. Zoöl.*, vol. 11, p. 365.
- Morgan, T. H. and Cattell, E., 1912 Data for the study of sex-linked inheritance in *Drosophila*. *Jour. Exp. Zoöl.*, vol. 13, p. 79.
- Stevens, N. M., 1905 Studies in spermatogenesis with special reference to the "accessory chromosome." *Carnegie Inst. Washington Publ.*, 36.
- 1908 A study of the germ-cells of certain Diptera. *Jour. Exp. Zoöl.*, vol. 5, p. 359.
- Sutton, W. S., 1902 On the morphology of the chromosome group in *Brachystola magna*. *Biol. Bull.*, vol. 4, p. 39.
- Wilson, E. B., 1905 The behavior of the idiochromosomes in Hemiptera. *Jour. Exp. Zoöl.*, vol. 2, p. 371.
- 1906 The sexual differences of the chromosome-groups in Hemiptera, with some considerations on the determination and inheritance of sex. *Jour. Exp. Zoöl.*, vol. 3, p. 1.
- Yule, G. U., 1911 *An introduction to the theory of statistics*. London.



Color Inheritance in Mammals

SEWALL WRIGHT

Reprinted by author's and publisher's permission from *Journal of Heredity*, vol. 8, 1917, pp. 224-235.

All of the emphasis during the early history of the field of genetics was placed upon the kind of researches dealt with in the first papers of this collection. The authors were concerned primarily with what was inherited and how. Their information came principally from the final stages of the organism's development, when it was fully adult, and all of its permanent characteristics were fixed and recognizable. All of this assumes a direct relationship between the gene and its characteristic expression, but the pathways the gene took in producing that expression had not been investigated, primarily because of

the lack of adequate tools and research methods to permit such studies. There was little question that the gene played an important part in growth and development, but as little information as to how it did it.

Wright takes advantage of the findings of biochemistry and histology in the development of his hypothesis concerning the production of coat color in animals. He investigates the actual mechanism of color formation, and indicates the mode of action of the gene in this process. This paper lays the foundation for the field of biochemical or physiological genetics, and in fact still remains as the basic source of information and the starting point of modern investigation. Wright continued his thoughtful investigations in this field for many years, and in 1941, he wrote a paper called "The Physiology of the Gene," published in Physiological Reviews, vol. 21, pp. 487-527. This paper is too long to reproduce here, but I recommend it most strongly to the reader.

One of the most significant events in the history of twentieth century genetics, in my opinion, is the shift in emphasis exemplified by this paper. The focus of attention on the ways the gene goes about doing what it does, rather than on the mechanics of its transmission plus its identification through its terminal expression, has given genetics a central position in the understanding and interpretation of biological phenomena. A thorough knowledge of the various modes of action of the gene will re-emphasize the unitary nature of the field of biology. The more we learn about the gene's mode of action the more obvious it becomes that the phenomena of embryology, biochemistry, physiology, evolution, and systematics—in fact, all major fields of biology—are for the most part only facets of this one common problem, and solution of one aspect of gene action is likely to have repercussions in several other diverse areas.

HEREDITY AS LOOKED UPON SINCE THE time of Weismann is relatively simple to understand. It consists merely in the persistence of a certain cell constitution (in the germ cells) through an unending succession of cell divisions. We see something of the mechanism, back of this persistence, in mitosis. We understand complications brought about by the reduction division and by the union of two germ cells at fertilization. Thus we no longer puzzle over the problem as to how an individual packs his characters into his reproductive cells, the problem which led to Darwin's theory of pangenesis. But if heredity seems simpler

than it did half a century ago, the problem of development has become more complex. We see clearly that development is no mere unfolding and growth of elements already present in the fertilized egg or even a sorting out of germinal rudiments for parts of the organism. The germ cell has a certain highly complex constitution, the adult organism another such constitution, and between the two is no simple one-to-one relationship. An almost infinitely complex series of interactions of elements must take place at each stage of development. The difficulty in the study of heredity is that the characters of the germ cell must be

deduced from a study of variation in characters at the other end of the developmental history. The wonder is that with such a method it has been possible at all to demonstrate unit variations in the germ cell. One of the most remarkable results of recent genetic work has been the detailed correlation of these hypothetical characters of the germ cell with structures actually observed there.

It remains for genetics to assist embryology and biochemistry in filling in the links in the chain between germ cell and adult in specific cases. Variations of adult characters must be traced back through the contributing causes at each stage of development until, if possible, something is learned of the nature of the ultimate germinal factors involved and, on the other hand, the ramifying influences of unit variations in the germ cell must be traced forward through development. Probably the most favorable point of attack for such work is in color inheritance in animals and plants. A larger number of unit factors have been isolated than in other kinds of characters and are available for comparative study. Much progress has been made in the chemistry and mode of formation of many of the pigments, notably the melanins, with which we are chiefly concerned in the higher animals. The very fact that it has been relatively easy to isolate unit factors in work on color inheritance suggests that in this case the chain of processes between germ cell and adult may be relatively simple. Observations which indicate that melanin pigment is formed in the cytoplasm of cells by the secretion of oxidizing enzymes from the nucleus suggest that the chain may be very short indeed when it is remembered that genetic factors are probably characters of the chromosomes and that these seem to be

distributed unchanged from the germ cell to all other cells.

The present paper is an attempt to make as simple a classification of color factors in mammals, based on their effects, as possible, and to suggest a working hypothesis which will relate the biochemical knowledge concerning melanism with this classification and with certain peculiar relations between the colors. The writer wishes to emphasize, however, that for the present the chemical terms are used rather for the sake of giving a definite scheme to which genetic facts may be referred than for their own sake. Since the earliest work on color inheritance, many geneticists, notably Cuénot,¹ Castle,² and Little,³ have tried to give a physiological interpretation to their results. The hypothesis advanced here is based to some extent on their conclusions with modifications intended to bring under one point of view certain curious new facts.

COLOR IN MAMMALS

Melanin pigment is found in the skin, fur and eyes of mammals. The present paper will deal largely with the gross effects as our knowledge of the ultimate differences of the colors is still very unsatisfactory. Only mammals are dealt with, as in other classes on which genetic work has been done the pigment colors are largely masked by structural effects.

The most highly pigmented condition is found in the color black. The pigment granules in this case are not really black but a very dark sepia brown. White in mammals seems always to be a structural color found

¹ Cuénot, L., 1903. *Arch. Zool. Exp. et Gén.* (4), vol. i. Notes et Revue, p. 33.

² Castle, W. E., H. E. Walter, R. C. Mulenix and S. Cobb, 1909. *Carn. Inst. Wash. Publ.*, 114.

³ Little, C. C., 1913. *Carn. Inst. Wash. Publ.*, 179.

in the absence of all pigment. There are two distinct series by which black may be reduced toward white. There is first the type of dilution found in brown and tow-colored human hair, or in dilute black guinea-pigs, which reveals the sepia color of the pigment. A very different kind of dilution of black is to be seen in the so-called blue or maltese mammals—blue mice and rabbits, maltese cats, etc. The effect is somewhat similar to that in blue roans among horses and cattle and seems to be due to a similar cause on a finer scale. Blue roans have an intermingling of jet black hairs and white hairs while the maltese mammals have dense black pigment masses alternating with colorless spaces within the hair.

The colors which do not enter into either of these dilution series or their combinations are those which have a distinct orange-yellow tinge such as is seen conspicuously in red human hair, red and yellow cattle, bay, chestnut and dun horses, tan dogs, etc. The most highly pigmented colors of this kind are the so-called reds. The pigment granules appear orange-yellow in such hair, but it does not seem to be settled whether there is an essential chemical difference from sepia-brown granules or merely some structural difference. The appearance of the intense reds varies somewhat in different mammals but there seems little reason for doubting their essential similarity. Red undergoes different modes of dilution comparable to those described for black. Reduction to yellow or cream is comparable to the sepia type of dilution of black, while a more coarsely granular type of dilution comparable to maltese is found in light reds. These light reds are slightly redder in hue than the yellows of similar intensity. The two series may, of course, be combined.

Finally there are intergrades of various sorts between the different sepia and yellow series. A coarse-grained mixture gives the effect of bay, dun, or sooty yellow depending on the intensity of the colors. A finer type of intergrade seems to be present in the chocolate color of brown mice, rabbits, guinea-pigs and liver-colored dogs. These browns, however, are much closer to the sepias than to the reds and yellows and are not always distinguishable. Genetic evidence sharply distinguishes browns which are due to reduction of black toward white, and browns which are reductions toward yellow.

By combining the different kinds of dilution with the different kinds of intergrades between sepia and yellow an almost infinite variety of colors is produced, while the complex patterns in which these colors may be distributed make possible still further diversity in color effect.

Skin color in general corresponds roughly with hair color. The pigments in the eyes are like those in the skin and fur, but the appearance is generally much modified by structural effects. In dilute human eyes, for example, the appearance is blue, although the pigment is sepia brown. In the rodents the reflection from the back of the retina gives a red color when the pigment is reduced, which in the complete absence of pigment becomes pink as in albinos.

CHEMISTRY OF MELANIN

A large amount of work in the last twenty years has firmly established the hypothesis that melanin is an oxidation product of tyrosin or related products of protein metabolism. Enzymes have been extracted in a great many animals and plants, which have the power of oxidizing tyrosin and related substances to dark brown

pigments closely resembling the natural melanins. Studies of Hooker⁴ on cultures *in vitro* of frog mesenchyme indicate that the pigment granules are formed in the cytoplasm immediately surrounding the nucleus, presumably under the influence of oxidizing enzymes secreted by the latter.

The nature of the differences between colors is still far from clear. Onslow⁵ could find no chemical differences between pigments extracted from black and yellow rabbits and considers that they differ merely quantitatively. Most others do find chemical differences as well as physical ones. Black pigment seems always to be granular, while red may be either granular or diffuse. Lloyd-Jones⁶ found only granules in both intense and dilute black pigeons, and found red granules in red pigeons, but merely a diffuse yellow in the dilute yellows. The Davenports⁷ found granules in brown and black human hair, but merely a diffuse color in auburn hair.

There is a distinct difference in solubility between the dark colors and yellow. Miss Durham⁸ found that yellow granules in yellow mice dissolve quickly in potash, brown less quickly, and black not at all. Gortner⁹ was able to separate two pigments in black wool—one easily soluble in very dilute alkali and also soluble in dilute acids, while the other dissolved only slowly in alkali and not at all in dilute acids. This, of course, is a chemical difference. The acid-soluble type was

of protein nature. He considered it to be a diffuse coloration of the keratin structure, while he identified the insoluble type with the granules. In red human hair he found only the acid-soluble type. In brown horse hair and black wool he found both, while in brown and black human hair, black rabbit hair and black feathers of domestic poultry and crows he found only the insoluble type. His two types evidently correspond more or less closely with yellow and black pigment. This difference in solubility does not necessarily indicate that yellow and black are produced from different chromogens. Indeed, Gortner has shown that the insoluble type may be produced from the soluble by treatment with strong alkali. In a brief paper in 1912, however, Gortner¹⁰ reports on a more fundamental difference between the insoluble melanin from black feathers, black rabbit hair and brown horse hair, and the soluble melanin from black wool and brown horse hair. The former yielded some 3% ash consisting largely of iron oxide, while the latter yielded little or no ash. This seems to demonstrate a difference in chromogens in at least these cases. But even here black pigment may consist of a mixture of melanins containing iron and melanins which do not, derived perhaps from the same chromogens as those which under other conditions produce yellow.

The chemical difference which Gortner found suggests that the presence of iron bearing chromogens may be the thing which is determined by Mendelian factors for black as opposed to yellow. Such a view, however, is not in harmony with certain other results. Miss Durham¹¹ found

⁴ Hooker, D., 1915. *Anat. Rec.*, 9:393.

⁵ Onslow, H., 1915. *Proc. Roy. Soc.*, B-89: 36.

⁶ Lloyd-Jones, O., 1915. *Jour. Exper. Zool.*, 18:453.

⁷ Davenport, C. B. and G. C., 1909. *Amer. Nat.*, 43:193.

⁸ Durham, F. M., 1904. *Proc. Roy. Soc. London*, 74:310.

⁹ Gortner, R. A., 1911. *Biochem. Bull.*, 1:207.

¹⁰ Gortner, R. A., 1912. *Proc. Soc. Exp. Biol. and Med.*, 9:3.

¹¹ Durham, F. M., *loc. cit.*

an enzyme difference in the skins of black and yellow guinea-pigs. Onslow¹² was unable to confirm Miss Durham's results, but also found an enzyme difference. He was able to extract a peroxidase from the skins of black rabbits but not from yellow rabbits. It seems unlikely that the same factor should determine both the presence of a particular chromogen and of a particular enzyme. One way to reconcile Gortner's and Onslow's results is to suppose that a feebly acting enzyme oxidizes certain chromogens in the cell giving the appearance of yellow. In the presence of a more powerful enzyme these chromogens and also others (including some containing iron) are thoroughly oxidized yielding sepiu granules. Such a view fits in excellently with our knowledge of the relations of the colors in heredity.

The processes which yield black and yellow are not independent of each other. Both may be reduced or inhibited by the same factor. Onslow investigated several such cases in rabbits. He was able to extract peroxidases from the skins of black, blue and gray rabbits which in the presence of hydrogen peroxide would convert tyrosin into dark pigments. He was unable to extract such enzymes from the white parts of Dutch rabbits and from albinos, both recessive whites. In both cases (as well as in the case of yellow rabbits) the addition of tyrosinase from another source to the solution of extract and tyrosin enabled pigment to develop. As the Dutch pattern and albinism affect yellow and black stocks of rabbits alike, it is evident that our feeble enzyme for yellow and the powerful one for black must contain some common element the loss of which prevents either kind of pig-

mentation. Thus in different animals of a stock or in different areas on the same animal black and red tend to be intense, dilute or absent alike. But in the same area, there is, in general, a reciprocal relation. Black and red, it is true, may be present together as in reddish-brown human hair and brown horses, but in most cases black obviously increases at the expense of red. This demonstrates either a common chromogen or a common enzyme element or both in the production of black and yellow. More will be said of these relations later.

We have noted several very different kinds of color variations which Onslow has shown to be due to hereditary differences in the enzyme element of the reaction, viz., recessive yellow, albinism, and a recessive white pattern. In two other kinds of variation he obtained a result similar in this respect. In the white parts of English rabbits and in the white belly of gray rabbits—the former due to a dominant factor for white pattern, the latter to a dominant factor for yellow pattern (the agouti factor)—he was not only unable to extract oxidizing enzymes, but found positive inhibitors to be present which prevented pigment production when peroxidases from other sources were added. Gortner¹³ has shown that certain chemicals actually have an inhibitory effect on the reaction of tyrosinase with tyrosin and suggested the bearing on the problem of dominant whites. The point of most interest here is that color variations of nearly every kind have been shown to be due genetically to variations in the enzyme element of the reaction which produces pigment.

HYPOTHESIS

The chemical and histological in-

¹² Onslow, H., *loc. cit.*
¹³ Gortner, R. A., 1911. *Jour. Biol. Chem.*, 10:113.

vestigations indicate: first, that melanin is produced by the oxidation of certain products of protein metabolism by the action of specific enzymes; second, that this reaction takes place in the cytoplasm of cells probably by enzymes secreted by the nucleus; third, that various chromogens are used, the particular ones oxidized depending on the character of the enzymes present; and finally that hereditary differences in color are due to hereditary differences in the enzyme element of the reaction. The following provisional hypothesis is built around these considerations:

First, we will suppose that color depends on the rates of production or of potency of two enzymes. Enzyme I is essential to the production of any color, but by itself only produces yellow. Enzyme II is supplementary to enzyme I, producing no effect by itself. The compound enzyme, I-II, produces a darker kind of pigment than enzyme I alone, viz., sepia. Enzyme I-II is also more efficient than

enzyme I in another way. It produces sepia pigment even when enzyme I is at too low a potency to produce any yellow by itself. Above the level at which enzyme I produces effects, the two enzymes, I and I-II, compete in the oxidation of chromogen. Chromogen which is oxidized by enzyme I to yellow pigment is incapable of further oxidation to black. In the mixture the presence of the relatively pale yellow color serves mainly to dilute the color of the hair. This production of yellow reduces the amount of dark pigment and the apparent intensity of color, both by reducing the amount of enzyme I which can unite with II to form the enzyme for black production and also by using up chromogen which would otherwise become black. That tyrosinase is exhausted in the production of pigment has been shown by Gortner,¹⁴ who also quotes experiments by Roques (1909) to the same effect.

¹⁴ Gortner, R. A., 1912. *Proc. Soc. Exp. Biol. and Med.*, 9:1.

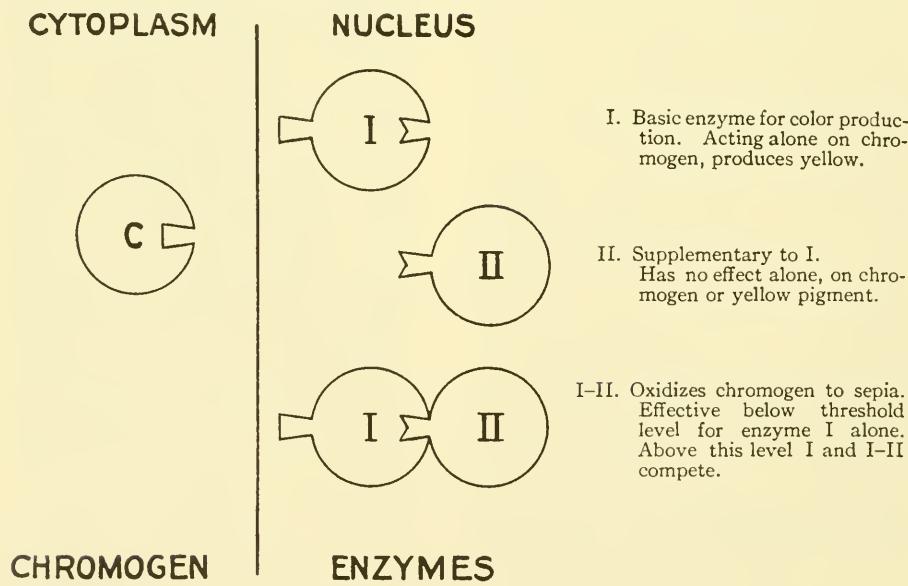


Fig. 1.

There are three points in the diagram (Fig. 1) at which physiological processes may affect color-production independently, viz., by influence on chromogen, on enzyme I, and enzyme II. In the first two cases color should be modified regardless of its quality. But as there is as yet no experimental evidence that genetic factors determine variations in the chromogen element it will be convenient at present to consider all such cases as due to influence on enzyme I.

The relations between black, red and white in this scheme are obvious. Inhibition of enzyme II when enzyme I is present gives red. Inhibition of enzyme I gives white regardless of whether enzyme II is present or not. The intergrading colors must be looked upon as resulting from reductions of various kinds in the activity of the enzymes. We have recognized two distinct types of intergrades between black and red, the coarsely granular type of bays and sooty yellows, and the finer type of chocolate browns. A mere reduction in quantity of enzyme II may perhaps be thought to result in an intermingling of black and red granules as one or the other of the respective enzymes chances to predominate, while a uniform reduction in potency in some other way not involving a reduction in quantity may give the chocolates and the pale browns of the pink-eyed rodents, in which there seems to be little more tendency for red to win in competition than in intense blacks. Similar relations with respect to enzyme I may account for the differences between the maltese and sepia types of dilution of black and the correlated light red and cream types of dilution of red. Of course, any such definite assignment of physiological effects to factors is at present to be taken mainly as a means of visualizing their action in our igno-

rance of the real physiology. In order to make wholly clear the relations supposed to hold between the different colors on the hypothesis, the accompanying diagram (Fig. 2) is given. Full quantity is represented by two symbols, reduced quantity by a single symbol, reduced potency by a symbol of small size, and complete inhibition or impotency by absence of symbol. Variations in enzyme I are given horizontally, variations of enzyme II vertically.

The classification of color factors which it is desired to present is based primarily on the difference between factors which act as if on enzyme I, and those which act as if on enzyme II. A secondary classification is based on the mode of action. There is a wide difference between factors which produce no effect in parts of the coat though with maximum effect in other parts, *i.e.*, bring out a pattern, and factors which produce the same effect throughout fur, skin and eyes. In the former case increase in the array of factors causes extension of the pattern; in the latter whatever pattern may be present tends to be stationary on increase in the array of factors. There is instead a further general change in intensity. The coarsely granular types of intergrades should perhaps form a third subclass in each main class, but for the present they are most conveniently put with the pattern factors. They can be considered as determining a fine pattern within the individual hairs.

CLASSIFICATION OF COLOR FACTORS

1. Factors which affect distribution and intensity of color, largely irrespective of the kind of color. (Act as if on enzyme I.)

- (a) Factors which affect the distribution of color in contrast with white.

(b) Factors which affect the intensity of color in all colored areas of the skin, fur and eyes.

2. Factors which affect the distribution and intensity of differentiation from yellow to black—effects, of

course, visible only in colored areas.
(Act as if on enzyme II.)

(a) Factors which affect the distribution of a dark color (black, sepia, brown, etc.) in contrast with a yellow.

(b) Factors which affect the inten-

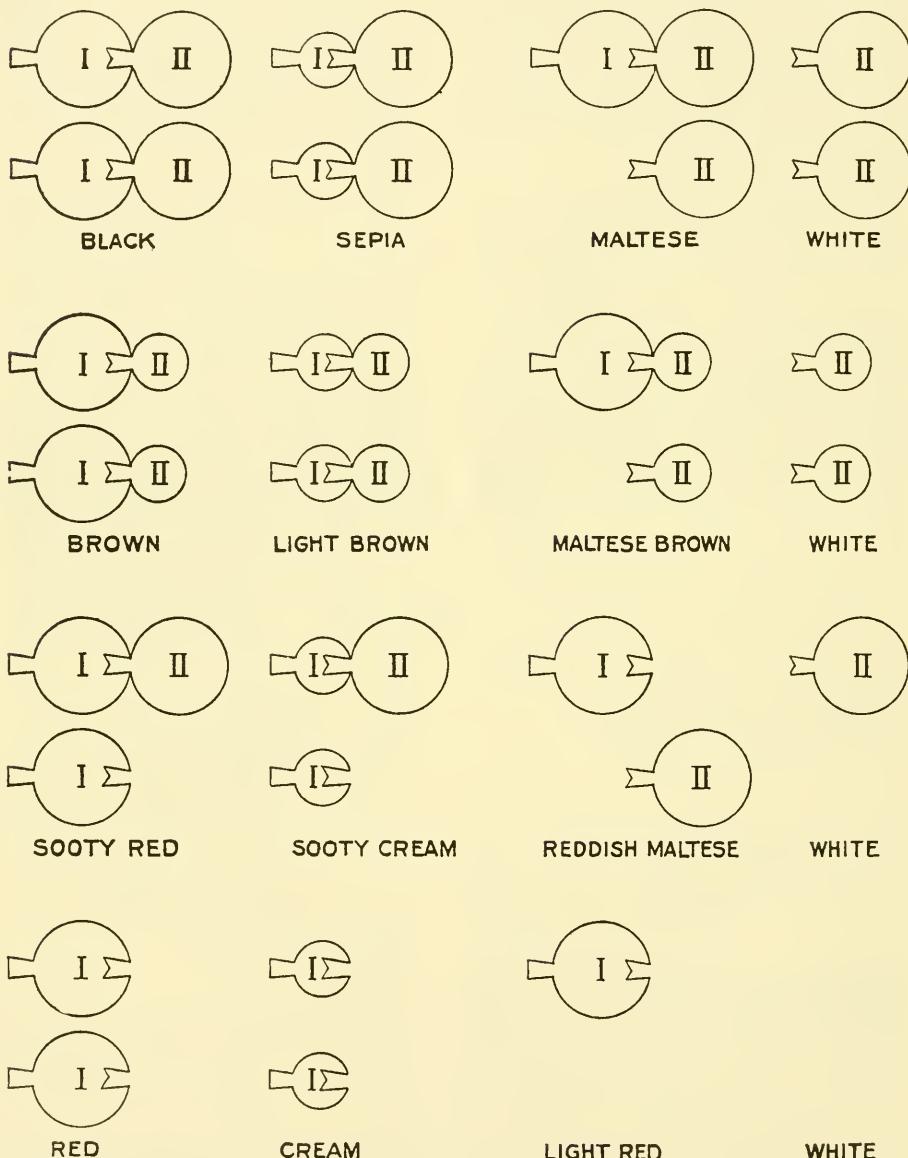


Fig. 2. Production of coat colors in mammals. The inherited color factors can be classified as if acting on one or other of two enzymes. For explanation see text.

sity of only the dark colors with effects visible wherever such colors develop in skin, fur and eyes.

This classification differs slightly from one previously advanced by the writer¹⁵ in that classes 2 and 3 of the earlier paper are brought more closely together as classes 2a and 2b.

As an illustration of the classes of factors, consider a guinea-pig which is like a solid black except for the following factors: $\Sigma_w C_d C_d AAbb$. Σ_w represents unanalyzed hereditary factors of class 1a which determine a pattern of white regardless of anything else. Factor A of class 2a puts a yellow band in each colored hair. Factor C_d of class 1b makes the yellow a dilute yellow instead of red, and makes the dark parts of the hair lighter than otherwise and perhaps slightly dilutes eye color. Finally factor b of class 2b further modifies the sepia in the coat and eye producing light brown but does not affect the yellow. The animal is a brown-eyed light brown agouti with yellowing ticking and a white pattern in the coat.

It is often difficult for one who is not working in the inheritance of coat color to understand just what color is supposed to be determined by a given array of factors. Probably this can be done most easily by considering the factors in the order just given. First are the factors of class 1a which determine patterns of color and white. No factors considered later can change these white areas. Next to be considered is the pattern of dark and yellow colors visible in the colored areas. These are determined by class 2a. Finally the kind of yellow in the yellow areas may be seen by noting the factors of class 1b and the kind of dark color in the remaining areas by a simultaneous

consideration of classes 1b and 2b. Eye color is generally determined wholly by these last two classes, but occasionally extreme white patterns of class 1a invade the eye.

DISCUSSION OF CLASSES OF FACTORS¹⁶

Class 1a.—White patterns are very common in mammals and most of them are obviously determined by factors of this class even where the mode of inheritance has not yet been thoroughly analyzed. The factors which determine the white face of the red Hereford cattle are a good example. The same white face appears in the black cattle from the cross of Hereford with Aberdeen-Angus. Evidently the factors involved strike at color in general regardless of its quality. The same is true of the different types of white patterns found in roan and in white Shorthorns, in the black and white Holsteins, and in Dutch belted cattle. The common white patterns of horses, dogs and cats are similarly independent of the ground colors of the animals. This seems to be true of the belt in Hampshire hogs, but is not so certain in other white patterns in hogs which may correspond to extreme dilution of yellow patterns. One or more recessive Mendelian factors have

¹⁶ Most of the statements in regard to color inheritance are based on well-known investigations. A very detailed review of the literature to 1913 is given by Lang, 1914 (*Experimentelle Vererbungslehre*, pp. 467-888). A discussion of our present knowledge on the subject with extensive bibliography is given by Castle, 1916 (Genetics and Eugenics). The writer may say that all of the statements in regard to guinea-pigs can be based on his own experience, and he has also had the opportunity of becoming directly acquainted with the mode of inheritance of most of the color varieties among rats, mice and rabbits as an assistant in Professor Castle's laboratory for three years. Only a few references are cited in this paper.

¹⁵ Wright, S., 1916. *Carn. Inst. Wash. Publ.*, 241, part II.

been demonstrated for the patterns of piebald mice, hooded rats, and Dutch rabbits, while a dominant factor is responsible for the English pattern of rabbits and the white blaze in man.

The mechanism by which such patterns are determined is interesting to speculate upon, but very little is yet known. In the case of the English rabbit, Onslow has demonstrated the presence of an inhibitor which prevents the oxidation of tyrosin by tyrosinase. Apparently the power of a cell to produce this inhibitor is determined by a variety of conditions of which the level of the English factor (absent, heterozygous or homozygous) is one and differences brought about in regional differentiation another. As a result of the combined effects of these conditions a given cell either has no power to produce the inhibitor or can produce sufficient to inhibit any intensity of color. The extent of white patterns seems to be the same in general whether the ground color is intense or dilute. In Dutch rabbits Onslow found no enzyme inhibitor but simply an absence of peroxidase. Here we must suppose that some essential condition in the cells for production of enzyme I is determined by the array of recessive white pattern factors in conjunction with regional differentiation.

The maltese type of dilution which appears under the microscope in such a case as the blue rabbit as due to an alternation of colorless spaces with intense pigmentation within each hair is put provisionally in class 1a. Recessive factors which determine simultaneously maltese dilution of black and the homologous kind of dilution of yellow have been demonstrated in dogs, cats, mice and rabbits.

Class 1b.—Correlated dilution of black and yellow is probably very

common in mammals. A case which seems to be dominant is found in the factor by which dun, mouse and cream-colored horses differ from bays, blacks and chestnuts respectively. An imperfectly dominant factor differentiates dun and yellow cattle from blacks and reds.¹⁷ The sepia and yellow guinea-pigs differ from black and red ones by a unit recessive factor.¹⁸ There are two more allelomorphs of this dilution factor. In red-eyed dilutes yellow disappears entirely, giving place to white, and eye color becomes distinctly dilute while in the lowest recessives of the series—the albinos—sepia as well as yellow nearly disappears and the eyes become pink.

Rats show a series of three allelomorphs which seem to be comparable to the guinea-pig albino series.¹⁹ The lowest recessive determines complete albinism while the second member of the series is much like red-eyed dilution in guinea-pigs. Black is diluted, yellow is reduced to white and the eye color to red. Rabbits show a series of three allelomorphs in the complete albino, Himalayan albino and fully colored varieties. Albinism is found in many other mammals and always seems to be recessive in inheritance. There are a number of curious features in the albino series in guinea-pigs which have had much to do with shaping the hypothesis advanced here. The table below shows roughly the model grades of intensity of yellow and black fur and eye color found with each of the ten possible zygotic formulae. Guinea-pigs of these ten formulae can easily be distinguished by the results of crosses with albinos, the lowest recessives. The full evi-

¹⁷ Wilson, J., 1909. *Sci. Proc. Roy. Dub. Soc.*, 12:66.

¹⁸ Castle, W. E., and S. Wright, 1916. *Carn. Inst. Wash. Publ.*, 241.

¹⁹ Whiting, P., 1916. *Sci., n.s.*, 43:781.

dence has been given in a recent publication.²⁰

	<i>Yellow fur</i>	<i>Black fur</i>	<i>Black eye</i>
CC	Red	Black	Black
CC _d	Red	Black	Black
CC _r	Red	Black	Black
CC _a	Red	Black	Black
C _d C _d	Yellow	Dark sepia	Black
C _d C _r	Cream	Dark sepia	Black
C _d C _a	Cream	Light sepia	Black
C _r C _r	White	Dark sepia	Red
C _r C _a	White	Light sepia	Red
C _a C _a	White	Sooty white	Pink

There are a number of ways in which such a series of four allelomorphs, as C, C_d, C_r and C_a can be interpreted. Complete linkage could explain the mere ratios obtained in crosses but leaves wholly unexplained the graded series of physiological effects. Four diverse non-linear variations of a factor would be another possibility. It seems most in harmony with the broad facts of the series, however, to consider these as four levels in potency of some one factor. But with this interpretation two peculiarities of the series stand out at once. First, we get complete albinism in yellow parts of the fur at a level in the series (C_rC_r) at which black in fur and eye is still quite intense. This renders it unlikely that black results from the further oxidation of yellow pigment or the reverse. The hypothesis suggested is that the efficiency of enzyme I, the basic enzyme for color production but which produces only yellow when acting alone, is increased by union with enzyme II so that black pigment is produced at a much lower threshold than yellow. The second curious fact is the bimodal curve of intensity in black fur in passing down the series of zygotic formulae. If C_dC_a determines a higher potency of enzyme I than C_rC_r it would seem that it should de-

termine a higher intensity of color everywhere. Yet C_dC_a gives a distinctly paler black than C_rC_r but a more intense yellow and eye color. It will be noticed that this irregularity occurs just at the point at which yellow is able to appear and the explanation suggested is that competition of enzyme I with the enzyme for production of black, I-II, begins at this point and is able to reduce the intensity of the black produced. Both the available quantity of chromogen and the available quantity of enzyme I are reduced in the production of the relatively pale yellow color. In the eye no factor ever brings out yellow, and perhaps enzyme I is at a much lower level than in the fur.

The different thresholds of black and yellow are attested by a great number of facts. In the dilute rats and Himalayan rabbits as well as in the red-eyed dilute and albino guinea-pigs, black is able to develop but not yellow. In many animals with black and red phases, white patterns appear in the red phase which are absent in the black phase. The white belly of the red fox contrasted with solid sepia of the silver phase is an example. A similar example in rabbits will be discussed later. Again climatic changes in pelage follow the same law. Squirrels and hares lose the yellow in their fur in winter before they lose black. Examples of the competition between black and yellow are discussed under class 2a.

Class 2a.—Factor differences of this group are very common. The factors by which red cattle and swine, bay horses, tabby and yellow cats, bicolor and red dogs; agouti, tortoise and yellow rodents, differ from the blacks in each species are examples.

The agouti factor of rats, mice, guinea-pigs, rabbits, cats, and other animals is an interesting example. In

²⁰ Castle, W. E., and S. Wright, *loc. cit.*

all cases agouti is dominant over the absence of the pattern in blacks, browns, etc. Onslow was able to extract a substance which inhibits the oxidation of tyrosin by tyrosinase, from the white belly of gray rabbits, where the agouti factor removes all black from the hair. Onslow compared the case with the enzyme inhibitor which he found in English rabbits but on our interpretation, the latter was an inhibitor of enzyme I, the former of enzyme II. Since only one genetic factor is involved, it seems likely that the same cause which modifies a solid black rabbit in such a way that yellow ticking appears on the back is responsible for the white belly. According to the hypothesis presented here, white appears on the belly when black is inhibited not because there is a general inhibition of pigment production but because enzyme I is below the threshold for the appearance of yellow. There is a parallel case in the white bellied agouti mice. In this case, however, it is possible to increase the general intensity of pigmentation in the animal so that yellow appears on the belly. In agouti guinea-pigs the belly is normally yellow but paler than the back. Such white patterns as those of the gray rabbit and white-bellied agouti mouse illustrate the possibility of confusing white patterns due really to yellow pattern factors of class 2a acting where enzyme I is below the yellow threshold with white patterns of class 1a. It is likely that many dark-eyed whites among mammals are of the former kind. The polar bear is probably an example.²¹

It has been mentioned that the extent of white patterns seems to be independent of the intensity of color due to enzyme I. A parallel statement

cannot be made of the agouti pattern. The latter is greatly modified in extent by conditions which have differential effects on the production of black and yellow. In pink-eyed guinea-pigs with a much reduced potency of enzyme II, the agouti band is greatly widened. On the other hand, the agouti band in ordinary intense guinea-pigs can be greatly reduced by crossing with exceptionally intense blacks. Punnett's density factor in rabbits²² eliminates the agouti pattern altogether. It is also more effective in the ordinary black agoutis than in brown agoutis. Thus the agouti factor seems to determine a certain quantity of inhibitor which is not as a rule sufficient to eliminate all black and the effect depends not merely on the level of the agouti factor but also on the level of potency or quantity of the substance to be inhibited. The dominant agouti factor (A) and the recessive factor of sooty yellow rabbits (e) make an interesting contrast between two factors of class 2a. In the former case, as we have seen, Onslow demonstrated that an enzyme inhibitor was produced, in the latter he was simply unable to demonstrate peroxidase, indicating a reduced quantity or potency as compared with blacks. The following table shows the effects produced by these factors when added to those of a solid black rabbit (aaEE):

	<i>aaEE</i>	<i>AAEE</i>
Back	Black	Black ticked with yellow
Belly	Black	White
	<i>aeee</i>	<i>AAee</i>
Back	Sooty yellow	Clear yellow
Belly	Black	White

The case can readily be understood if we suppose; first, that both enzymes I and II are strong on the back

²¹ Lang, A., 1914. *Experimentelle Vererbungslehre*.

²² Punnett, R. C., 1912. *Jour. Gen.*, 2.

but feeble on the belly in all rabbits due to a regional differentiation. We will suppose that enzyme I is below the yellow threshold on the belly. Second, we will suppose that factor A determines the production of an inhibitor with the same subtraction effect on enzyme II everywhere, while factor e determines a general proportional reduction in rate of production of enzyme II. On this basis it follows that factor A produces a partial inhibition of black on the back, revealing yellow but a complete inhibition of black on the belly where, however, only white can be revealed. Factor e reduces black on the back sufficiently to permit yellow to predominate in competition while on the belly, where there is no competition from yellow, what little of the black producing enzyme I-II is produced is fully effective and black, or at least blue, results.

Class 2b.—The factors which reduce the intensity of black areas in skin, fur and eyes without affecting red areas form a clearly defined class. The brown-eyed chocolate mice, guinea-pigs and rabbits; the pink-eyed pale sepia or "lilac" mice, guinea-pigs and rats, and the red-eyed rats of similar coat color²³ differ from blacks by factors of this class. Among the larger animals the difference between liver-colored and black dogs seems to be of this kind. Probably chestnut and liver-colored horses differ from bays and blacks by such a factor. All of these factors are recessive. In the pink-eyed mice, guinea-pigs and rats it is remark-

able to what an extent black is diluted without bringing out any distinctly reddish tinge although in red regions of the fur as in the agouti band of gray varieties the red appears in full intensity. There is evidently a different sort of reduction of enzyme II from that in the sooty yellow rabbit. A normal quantity of enzyme II but reduced potency in some other way would seem to be required in class 2b.

SIMULTANEOUS EFFECTS

It has been assumed so far that factors act only on one or the other of the hypothetical enzymes I and II. In the great majority of cases this is satisfactory but it is not impossible that a factor may influence both enzymes in the course of development. In fact the writer will soon publish evidence on one such case in guinea-pigs. Tri-color male guinea-pigs of many different stocks agree in showing a slightly greater average area of color as opposed to white than their sisters, and they also show relatively more black as opposed to red. Maleness seems to determine a higher level of both enzymes I and II as regards pattern. The effect on color is perhaps due rather to a general metabolic difference in the cells of males and females early in ontogeny than to any specific modes of action on the two enzymes. The same may be true of certain coat patterns in which it seems necessary to suppose that the level of enzymes I and II is raised or lowered simultaneously in some respect by regional differentiation. We have cited the cases of the rabbit, mouse, and guinea-pig in which it was found convenient to suppose that both enzymes I and II are strong on the back and weak on the belly. Similar cases are common. Another sort of example seems to be present in the tiger pattern of cats. In yellow cats the pattern is shown by

²³ These red-eyed rats first described by Castle (1914, *Amer. Nat.*) under the name of yellows must not be confused with the red-eyed rats described by Whiting (*loc. cit.*). In the former yellow pigment is unaffected and to a large extent gives the color to the fur in agoutis, owing to the great reduction of black. In the latter, yellow is reduced to white, while black is only slightly affected.

alternate orange and cream stripes which on our interpretation must indicate alternate stripes of high and low potency of enzyme I. In tabby cats, the intense stripes are solid black, the pale stripes show yellow ticking which seems to require that enzyme II also be strong in the former, weak in the latter.

CONCLUSION

In the present paper an attempt has been made to relate the findings of the biochemist in regard to melanin pigment with the great mass of curious relations between colors which have come to light in genetic work. A scheme is given which is designed to show the inter-relations of the different mammalian coat colors and a classification of color factors is suggested. It is hoped that these will be of use in organizing our present very extensive knowledge of color inheritance and in aiding in the discovery of new facts, or at least in leading to a better scheme

and classification.

Finally the bringing under one point of view of biochemical and genetic facts would have great intrinsic interest. The present paper attempts merely to trace the character—coat color—back one stage in development. Instead of considering factors as acting on this one character, they have been divided into two sets acting on two characters, production of the hypothetical enzymes I and II. Suggestions have been made in certain cases in regard to further tracing back of the action of the factors. A more thorough comparison than has yet been made of the effects of factors in all combinations should yield much data bearing on the process of pigmentation and give a very much more complete understanding of the heredity of color than we have at present. By constant comparison of the deductions from such work with the findings of the biochemist, it should be possible in the end to establish a very pretty correlation of results.



Unit Character Variation in Rodents

L. C. DUNN

Reprinted by author's and publisher's permission from *Journal of Mammalogy*, vol. 2, 1921, pp. 125–140.

By the time this paper by Dunn was written, it had become apparent that many of the visible variations that occurred in laboratory animals could be accounted for by assuming the existence of minute variations in their hereditary materials. These germinal variations, called mutations, became an important part of the Darwinian theory of evolution, after a period during which they were thought to

demonstrate it to be invalid (see Johannsen, p. 20). The application of the mutation theory to the evolution of natural populations, however, rested solely on hypothesis and extrapolation, until the appearance of Dunn's study. Dobzhansky, in "Genetics and the Origin of Species" (1941, p. 60) says, "Dunn . . . was the first to apply the above genetic interpretations to the aberrant individuals in wild species. . . . The aberrations found in nature resemble well-known breeds of domestic species (rabbits, mice, and guinea pigs), in which these characteristics are inherited as Mendelian recessives."

The paper, then, might be regarded as the beginning of efforts to utilize the knowledge gained from laboratory genetics in the interpretation of phylogeny and relationships between natural populations. It demonstrates the applicability of genetic theory and evidence in the explanation of evolutionary events, and this has become one of the major contributions to biology to arise from the study of genetics.

RECENT PROGRESS¹ IN THE STUDY OF the inheritance of coat colors in several species of rodents has revealed a rather striking similarity in the variations which have arisen in distinct species of that order. This similarity is not only a matter of appearance, which is familiar to all students of mammals, but extends as well to the manner of inheritance, and most recently has been found to characterize the localization of the determinants or genes for similar variations in two species. Such identity of cause of the same variation in two or more species indicates that such variations are homologous, and that the species which give rise to them have a relationship of a somewhat different and more intimate kind than that implied in the theory of relationship by common descent.

¹ In this paper, which is to be regarded as a cursory survey leading to a consideration of one or two special points rather than as an authoritative exposition of variation in rodents, I have not felt it essential to furnish a detailed bibliography. The necessary references may be found in Castle (1920) and Morgan (1919) as noted in the bibliography, and an excellent survey in Wright (1917), which also considers the physiological and chemical aspects of color variation and inheritance.

Before detailing the conditions in the species of rodents which have been studied, some explanation of the evidence and reasoning which underlie the localization of genes is due to the general reader. It is probably recognized by all students of biology that heritable variations arising generally by mutation are transmitted to the offspring in accordance with certain definite rules, known familiarly as Mendel's laws of inheritance. The chief of these laws states that heritable characters are transmitted as discrete units which segregate in the formation of the germ cells. A second principle asserts that the segregation of the units is independent, resulting in a random distribution of the characters of the parents among their gametes, such that when two units are involved the chances are equal that they will go together or separately. The first principle probably applies to all inheritance. Evidence has been brought forward to show that the factors or genes which represent the visible characters are not discrete but variable, and capable of change by selection, but this evidence has now been found to indicate not variability but plurality of units. The

second principle still applies to most cases of inheritance but has been modified by the finding that two or more characters may not always be distributed independently but when entering a cross together may tend to stay together, and when entering a cross separately may tend to remain separate (in different individuals) in inheritance. This peculiarity was first remarked by Bateson (1906) in the case of the inheritance of flower color and pollen shape in sweet peas. In his experiments purple flower (as opposed to red) and long pollen (as opposed to round) appeared to be associated or coupled in crosses so that a marked distortion was evident in the second generation ratio of 9:3:3:1 expected on the usual hypothesis of independent assortment, in favor of the classes (purple-long and red-round) representing the grandparental combinations of these characters. The opposite phenomenon was noted and named repulsion. Later, Morgan in 1910 found the same phenomena while studying inheritance of certain characters which had arisen by mutation in the vinegar fly (*Drosophila melanogaster*). He conceived these two exceptions to Mendel's principle of independent assortment as two aspects of a single phenomenon which he termed *Linkage* or associated inheritance.

The interpretation of these events has constituted one of the great advances of biological science. Sutton, in 1902, suggested that the marked parallelism between the discreteness and assortment of unit characters and the behavior of the chromosomes might be due to the residence in the chromosomes of the determinants or genes representing unit characters. Immediately after Bateson's announcement of coupling, Locke (1906) pointed out the similarity between this new mode

of inheritance and the results which might be expected if the coupled characters were determined in one chromosome. The development of this hypothesis, its proof and very important extension and generalization, have been the work of the American biologist T. H. Morgan, and of research workers associated with him, assisted more recently by data gathered by geneticists and cytologists working on many species of plants and animals. There has resulted from this work the elaboration of the chromosome theory of heredity, for the details of which, and the supporting evidence, the interested reader must be referred to the original works, especially as summarized in two publications of Morgan and his co-workers (1915 and 1919).

For our purpose it is sufficient to note in brief that the theory supposes that the differential representatives of heritable characters are located in the nuclear material of the egg and sperm cells, more precisely in those remarkably constant and individual organizations of chromatin known as chromosomes, which appear at the time of cell division and which probably retain their individuality even in the resting stages of the nucleus. Of the evidence it must be observed that the interpretation and proof of the theory rest entirely on the study of linkage, or associated inheritance. This phenomenon is observed in the tendency which characters exhibit of remaining through several generations in their original combinations, resulting in an alteration of the expected Mendelian ratios based on independent assortment. This tendency may be absolute, in which case linkage is said to be complete. More often it is partial, that is, characters originally associated may separate in a certain proportion of instances, or characters originally sepa-

rate may become associated. This change in the relationships of genes is known as "crossing-over" and it provides a quantitative measure of the strength of the tendency toward association. In terms of the chromosome hypothesis it is interpreted as an interchange of parts and of the genes which the parts carry, between two members of a chromosome pair, so that two genes originally resident in one chromosome may come to lie in two chromosomes and may thence be distributed to separate gametes and exhibit their effects (unit characters) in different individuals. For any two characters the number of times crossing-over occurs is found to have a characteristic value and this value is stated as the percentage of times crossing-over occurs as evidenced by the frequency of individuals possessing the two characters in the new combination. One other important aspect of these measurable breaks in linkage is that from the linkage strength may be inferred the proportional distance apart of linked genes. From cytological evidence crossing-over is supposed to take place between homologous chromosomes in the hybrid at the time when these chromosomes are intimately twisted one about the other. Breaks resulting in a separation of characters are then supposed on mere physical grounds to be more frequent between genes located far apart in the chromosomes than between those located near together. The bulk of the evidence indicates that the loci of genes are on the same straight line in any chromosome. Numerical strength of linkage may then be a measure of the exact localization of the genes in the germ plasm, and it is to a consideration of this point that our whole discussion has led. For if the genes for unit characters can be thus localized,

a direct comparison of species in which similar variations occur can be made on this point alone, even though the species cannot be crossed.

The study of localization of the genes for unit character variations is attended by numerous limitations. It can only be prosecuted through the experimental breeding of large numbers of organisms, exhibiting numerous variations. It is dependent even under these conditions on the occurrence of linkage, which is by no means common. It is a corollary of the location of genes in chromosomes, that the numbers of groups of linked genes be equal to the number of chromosome pairs present. Where the number of chromosomes is large, and the number of unit variations known is small, the chances are few that any two characters will be found to be localized in one chromosome pair. Even under such limitations, linked genes have been studied in several insects (chiefly *Drosophila*) and plants, and most recently in mammals. The general results of these studies have been to confirm the chromosome theory and to increase our knowledge of the localization of genes.

Correlative evidence has come from a brilliant series of cytological investigations on the germ cells of several organisms. It has been established that in the cells of each species are to be found a definite number of chromosomes, characteristic for the species. This number in germ cells is half the number found in the somatic cells, due to the intervention of reducing cell divisions. The chromosomes themselves are in general arranged in pairs of homologues in the somatic cells and in the primordial germ cells, one member of each pair having come from each parent, and this duality again becomes evidenced in the passage of

one member of each pair into the germ cells which form the next generation. The individual chromosomes are sometimes recognizable by peculiarities of shape, etc. More often their constancy is of numbers only. These cytological results have been made possible only by a high development of technique and can provide even when greatly extended only correlative evidence on the localization of genes. The geneticist or cytologist no more expects to behold the gene of which his literature is full than the chemist hopes to see the atom of which he speaks with unabated glibness. The gene remains useful as a concept and a notation, doubly so now that it includes an idea of spatial definition.

The above is a somewhat pretentious introduction to a discussion which adds so little to the matters mentioned in the opening paragraph, and yet I hope it has not been without interest to those engaged in the study of mammalian variation and evolution. The facts and theories discussed are to have an important place in general biology, and one may perhaps wish to hear of progress in a field which has tended at times to shut itself off from its fellow branches, by the dialect it has been forced to use.

Perhaps the best way of presenting the evidence on unit variation in color in the Rodentia is to describe the appearance and genetic behavior of each of the principal variations with a short list of the species in which it has been studied, and of the species in which a variation of similar appearance has been reported.² Where the inheritance of a variation has not been determined

by experimental breeding this fact is noted by an asterisk. This list makes no claim to completeness except in the cases of variations which have been studied experimentally. The rest of the variations have been reported as occurring in the wild or are represented by specimens in the Museum of Comparative Zoology at Harvard University, the Museum of the Boston Society of Natural History, or the American Museum of Natural History of New York. I am indebted to Dr. Glover M. Allen of the Boston Society of Natural History for help in gathering this part of the material, and for helpful suggestions and criticism of this paper.

All of the variations listed appear to have arisen, probably by mutation, from the primitive coat color of all rodents, the dull protective grey pattern known as "agouti." This color, which is actually a mosaic, is due to the presence of three pigments, black, brown and yellow, distributed uniformly over the dorsal surface of the animal. Each dorsal hair is characterized in general by an area of black next to the skin in which brown granules are mingled and generally masked by the black, followed by a band of diffuse yellow. The apex of the hair is typically black. The belly is always of a lighter shade than the dorsum, due to a lesser concentration of black pigment and a wider area of pale dusky yellow in the hairs. The "agouti" coat is seen in a typical form in the familiar wild house mouse (*Mus musculus*), the common rat of this country (*Rattus norvegicus*), etc. It characterizes the wild type forms of all the species included in the following list.

ALBINO

From this wild type distinct graded losses of pigment have taken place,

² This proceeding may be expected to lead to some errors since similarity of appearance is not always evidence of similarity in germinal constitution, but in the absence of breeding data we must use the only criterion available.

the extreme of which is complete albinism, or entire absence of pigment, leaving the fur clear white and the eyes pink. The pinkness of the eye is due to the absence of pigment in the iris, which is typically colored by black or brown pigment granules, so that the blood in the capillaries on the retina is directly visible. This variation is to be sharply distinguished from "partial albinism," a term which has been applied, unwisely it now appears, to the occurrence of white spotting in animals whose eyes retain their full color. The color of the eyes is an important point of distinction between complete albinos and spotted animals. Cases of true albinism have been reported in nearly all the families of rodents. Data from only ten of the commonest families are given here, the families being listed roughly in the order of their relationship from the more primitive to the more specialized.³

<i>Leporidae</i> —	<i>Oryctolagus cuniculus</i> — European "rabbit."
<i>Sciuridae</i> —	* <i>Marmota monax</i> — Woodchuck.
	* <i>Sciurus hudsonicus</i> — Northern red squirrel. ⁴
	* <i>Sciurus carolinensis leucotis</i> —American gray squirrel.
	* <i>Tamias striatus lysteri</i> — Chipmunk.
<i>Muridae</i> —	<i>Mus musculus</i> —House mouse.

³ I have followed the older order of classification which includes the Leporidae in the Rodentia.

⁴ Through the kindness of Professor Barrows of the Michigan Agricultural College and Prof. W. E. Castle of Harvard University the writer has learned of the capture of a pair of albino red squirrels by A. E. Secord, of Wheeler, Michigan. Breeding experiments to test the inheritance of this variation were to have been attempted but expense and pressure of other work have prevented the writer from undertaking the project.

<i>Rattus norvegicus</i> — Common rat.
* <i>Microtus pennsylvanicus</i> — Meadow vole.
* <i>Fiber zibethicus</i> — Muskrat.
<i>Peromyscus leucopus noveboracensis</i> —Deer mouse.
<i>Hystricidae</i> — * <i>Erethizon dorsatum</i> — Canada porcupine.
<i>Caviidae</i> — <i>Cavia cobaya</i> —Guinea-pig.

The inheritance of the albinism has been studied in the rabbit, the house mouse, the house rat, the deer mouse and the guinea-pig. In all of these it is due to a gene which acts as a Mendelian recessive to full color. At the same (albino) locus in the germ plasm have occurred other mutations. In the rat, a change in this locus has produced both albino and its dominant allelomorphs ruby-eyed dilute, in which the reduction of the melanic pigments is visible in the generally lighter tone of black, coupled with a complete absence of yellow. In the guinea-pig three graded variations have occurred: (1) *dilution*, resulting in a reduction of all pigments; (2) *ruby*, resulting in the absence of yellow, and the further reduction of black and brown in fur and eyes to very light shades (probably homologous with the ruby variation in rats); and (3) *Himalayan albinism*, which determines the absence of yellow and the restriction of black and brown to the extremities, ears, nose, feet, and rump, while the eyes are pink. These three conditions are distinct in appearance, do not blend in crosses and are all alternative allelomorphs with full color and with each other. No complete albinism is known in the guinea-pig. In the rabbit two changes have taken place: Himalayan albinism (probably homologous with

the Himalayan albinism of guinea-pigs) and albinism. These are allelomorphic with full color and with each other; that is, crosses of full colored animals with albinos produce only full colored young and in the second generation only colored and albinos. The same is true of the cross colored \times Himalayan, while the cross Himalayan \times albino produces only Himalayan and in the second generation only Himalayan and albino. The occurrence of this variation in several species, its similarities in appearance and in inheritance, and finally the production at the same locus as indicated by allelomorphism of other similarly appearing variations indicate that the particular locus in the chromatin at which these mutations have occurred is common to a number of widely different species and although such a statement cannot be proved except by a study of linkage relations between this and other common loci, it seems very probable that albinism is homologous variation throughout the rodents and in the species studied is due to homologous genes.⁵

PINK-EYE

This name has been applied by geneticists to a unit character in rodents which is not a form of albinism, as the pinkness of the eye might indicate, but a distinct eye and fur character. Animals exhibiting this variation show a general quantitative reduction in the black and brown pigments in

⁵ Since this paper was written, a fourth allelomorph in the albino series in rabbits has been reported by Castle (*Science*, vol. 53, April 22, 1921, p. 387). This variation, now studied genetically for the first time, is known as "chinchilla" and differs from the wild gray or "agouti" coat color in the absence of yellow, and its replacement by white, and in the reduction of black to a slate blue.

both fur and eyes. A certain amount of pigment is present in the iris but not enough to obscure the blood color of the retina. Yellow pigment is not affected. Pink-eyed animals with the "agouti" coat pattern therefore appear yellow since the black bases of the dorsal hairs are a reduced slaty or bluish tint and are covered by the fully intense yellow parts of the hair. Black animals with this variation are slaty or bluish all over in mice and a dirty near-white in rats and guinea-pigs. Its distinctness from albinism becomes evident when pink-eyed colored animals are crossed with albinos. The first generation offspring in this case are all as fully colored as the wild type and if inbred produce full colored, pink-eyed colored, and albino young.

The variation occurs in the following species:

<i>Sciuridae</i> —	* <i>Marmota monax</i>
<i>Muridae</i> —	<i>Mus musculus</i>
	<i>Rattus norvegicus</i>
	* <i>Microtus pennsylvanicus</i>
	* <i>Fiber zibethicus</i>
<i>Caviidae</i> —	<i>Cavia cobaya</i>

Its occurrence in the species marked * is probable but is based only on museum specimens with the coat colors peculiar to pink-eyed animals. The eyes in the mounted specimens may or may not agree with the original.

Data on the localization of this variation are available in large numbers for mice, and in lesser amount for rats and guinea-pigs. In these species it is a simple Mendelian recessive to full color (dark-eye). In rats and mice it is certainly a homologous variation, in appearance, in inheritance and in localization, for a large amount of linkage data indicates that the genes for pink-eye and for albinism are located in the same chromosome and

at about the same relative distance apart. This localizes both of these genes in both species, and leads to some interesting conclusions and speculations which will be more fully considered later. In guinea-pigs there is incomplete evidence concerning the location of the gene for pink-eye but some data which Dr. Sewall Wright has kindly extracted from his breeding records and sent to me indicate that the locus of pink-eye is not in the albino chromosome but elsewhere. As we shall see, this may prove just as instructive concerning the homologies between species in germinal constitution as the more definite localization of the gene in rats and mice.

YELLOW

The self or solid yellow coat coloration in rodents appears to be divisible as to its cause into two different categories. In the first of these may be placed those yellow varieties which have arisen by a change in a gene governing the *extension* of black and brown to the fur, and the alternative (allelomorphic) condition of *restriction* of these melanic pigments to the eye, while the pelt is yellow. In the presence of this gene (*restriction* [r]) the melanic pigments are probably not produced in sufficient amount or to a sufficient intensity to invade the fur, leaving the residual yellow which is present in all "agouti" animals in possession of the whole extent of the hair. This gene is recessive to full extension and is distinct in its inheritance from the gene which determines the barring of each hair in the agouti pattern. Animals may possess the gene for yellow, with the gene for "agouti" or without it. "Agouti" yellows have much lighter bellies than non-agouti yellows. Restricted yellow occurs in the following rodents:

<i>Leporidae</i> —	<i>Oryctolagus cuniculus</i>
<i>Muridae</i> —	* <i>Microtus pennsylvanicus</i>
	<i>Peromyscus maniculatus</i>
	<i>gambeli</i>
	<i>Rattus rattus</i>
	* <i>Rattus alexandrinus</i> × <i>R. rattus</i>
<i>Caviidae</i> —	<i>Cavia cobaya</i>

The distinction between this yellow and the second type, about to be described, is made on grounds of the mode of inheritance only, for the appearance of the latter type is identical with that of the former. The pelt of the second type is yellow and the eyes are dark. The gene which differentiates it is however dominant over its allelomorphs agouti and non-agouti. This dominant yellow is known only in the house mouse and because of its peculiar mode of inheritance has been the subject of considerable research through a combination of genetic and embryological methods to which Castle, Little, Kirkham and others have contributed. Such investigations have established the following facts:

(1) Yellow house mice do not breed true but when bred together always produce yellow and non-yellow young in the ratio of 2:1.

(2) Litters from yellow by yellow are on the average 25 per cent smaller than litters from non-yellow varieties.

(3) In the uteri of yellow females pregnant by yellow males there have been found disintegrating embryos approaching 25 per cent of the total embryos.

Ordinary heterozygotes (hybrids in one character) when bred together produce 25 per cent pure dominants, 50 per cent heterozygotes, and 25 per cent pure recessives. In the offspring of yellow mice the two latter classes have appeared in the expected proportions; pure dominant yellows have never been disclosed by breeding tests.

It has therefore been concluded that the 25 per cent missing from the litters, the 25 per cent of disintegrating embryos, and the 25 per cent of expected pure yellows are the same. The intrauterine death of this class has been supposed to be due to a recessive lethal gene which when received from both parents causes the death of the resulting zygote or individual. In every case this lethal gene has been transmitted with the gene for yellow. It may be either completely linked or identical with the gene for yellow. At any rate it is present at the same locus with the gene which determines yellow, and any individual which receives yellow from both parents receives likewise the lethal gene from both parents and is doomed to death before birth. As to why this combination of two lethal genes is fatal we are still in the dark.

This yellow gene and the lethal associated with it are known only in house mice,⁶ and the restricted yellow of the other species has not been reported in house mice. They are probably not homologous variations in spite of their similarity in appearance. The "yellow" varieties of rats are not really to be classified with other yellow rodents since they are actually "agoutis," differentiated from the wild gray by the pink-eye gene (to which we have referred) or by the very similar red-eye gene which act selectively on the melanin pigments to reduce rather than restrict them.

WHITE-SPOTTING

Almost as common as albinism among rodents is the spotting of cer-

tain portions of the coat with white. The white areas are as devoid of pigment as in albinos but here the likeness ends. Genetically white-spotting and albinism are distinct and contrary to the popular belief are not quantitatively but qualitatively unlike. Albinism is fundamentally the loss or change of a factor for the development of a peroxidase essential to the production of any pigment (cf. Wright '17) and its effects are of a general nature throughout the pelt and eyes. Spotting appears to be a change in a factor governing the distribution of the pigments in the pelage. When pigmentation is present all over the pelt the condition is known as self or not-spotted. Spotting is inherited independently of albinism, since certain albinos crossed with spotted animals throw only selfs, while other albinos derived from white-spotted colored stocks have given spotted offspring. An albino may therefore be genetically either self or spotted although unable to give evidence of this condition except in its offspring by a colored animal which supplies the gene for the development of color.

On the grounds of its inheritance white-spotting in rodents may be classified in three categories. The first of these is piebald or Dutch spotting, apparently due to a gene recessive to self coloration and probably independent of other coat color unit characters. It may thence be present with albinism, yellow, pink-eye, agouti or black (see below). Piebald animals may be characterized by a typical localization of the spotting in a belt or collar as in belted mice or Dutch rabbits; the spotting may be confined to the face ("white-face" mice), or it may be distributed in a fairly uniform dorsal pattern as in hooded rats.⁷ On

⁶ Several other factors may modify the appearance of yellow in mice; for instance, certain darkening factors in the presence of the yellow gene produce the black-and-tan and sable varieties of mice, while intensifying factors in the presence of yellow produce the brighter orange or red varieties.

⁷ A hooded *Microtus* has been noticed in the Museum of Comparative Zoology at Harvard University.

all of these types the white-spotting varies only within general limits. In other piebald mice and in guinea-pigs especially it appears purely at random, in irregular blotches hardly approximating any pattern at all. The spotting may also vary in amount from a few white hairs to over half the surface of the animal, although in general the pigmented areas exceed the white portions in total size. The belly is likewise more susceptible to spotting than the dorsum. This variation has been noted many times in wild species and I am certain the present list which has been hastily compiled does not represent the true distribution of this variation among rodents in general.

- Leporidae*— *Oryctolagus cuniculus*
- Sciuridae*— **Sciurus finlaysoni*
- Muridae*— *Mus musculus*
Rattus norvegicus
**Evotomys gapperi*
- Caviidae*— *Cavia cobaya*

In the rabbit, rat, mouse, and guinea-pig the similarity of its inheritance points toward a homology in this variation. Data on its localization are lacking except that in mice it is probably not located in the same chromosome with albinism and pink-eye, nor with the black-eyed white-spotting about to be discussed. In rats and guinea-pigs it is likewise not linked as far as is known with any other color variation, while in rabbits it may be a property of the same locus at which English or dominant spotting is determined.

The two other categories of spotting are peculiar each to a single species. The English broken spotting of domesticated rabbits is a Mendelian dominant to self-color and has no probable homologue in other species, while the black-eyed white-spotting of mice, likewise a dominant, is apparently peculiar to mice although wild rodents resembling this type have

been reported (e.g., *Sciurus finlaysoni*). This last variety is interesting in that it is less pigmented than any other type of spotting studied, some black-eyed white-spotted mice having pigment only in the eyes, while the rest of the pelt is pure white. It is discontinuous with piebald spotting, and is, like yellow, an unfixable hybrid, always throwing, when bred pure, a ratio of two black-eyed whites to one piebald. The cause of this peculiarity has lately been traced to its association with another lethal factor which determines the death in utero of all pure black-eyed white zygotes.

BLACK

One other color variation is common enough in rodents to make comparison profitable. This is the discontinuous change from the "agouti" coat to one which is black all over and it is probably due in all the species in which it occurs to a gene determining the exclusive development of black and brown pigments. It is always present in wild "agouti" type rodents and its appearance alone is conditioned by the change producing non-agouti, or the absence of the "agouti" pattern. Its recessive allelemorph is brown, which has occurred in rabbits, mice, guinea-pigs and possibly in rats. This gene is probably not linked with any of the other known genes in mice, but its relationships in other species have not been studied. The variation from agouti to black occurs in the following species:

- Leporidae*— *Oryctolagus cuniculus*
- **Lepus americanus*
virginianus—Eastern
varying hare
- Sciuridae*— **Sciurus hudsonicus*
**Sciurus niger ludovicianus*
**Sciurus niger niger*
**Sciurus carolinensis leucotis*
**Tamias striatus lysteri*

<i>Muridæ</i> —	<i>Mus musculus</i>
	<i>Rattus norvegicus</i>
	* <i>Fiber zibethicus</i>
<i>Caviidæ</i> —	<i>Cavia cobaya</i>

Specimens of individuals of other species which are much darker than the wild type are often seen in museums, usually labelled "melano" or "melanic variation." Many of these should not, I believe, be assumed to represent the true black variation. Some, as in "melanic" squirrels of various species, prove on close examination to be only very much darkened "agoutis" in which the black portions of each hair have been extended at the expense of the yellow portions. This variation has been studied by Punnett in the rabbit and found to be due to a gene distinct from black which is allelomorphic with the extension-restriction pair of allelomorphs. Much darkened "agoutis" have been produced in mice by crossing intense blacks or black-and-tans (a darkened form of yellow) with wild agouti-colored mice. The darkness is due in this case to a series of modifying genes distinct from either black, "agouti" or yellow which in the presence of these genes bring about an increase in the amount and intensity of the black pigment granules and a reduction in yellow. Such extended or darkened "agoutis" can usually (though not always) be distinguished from black by the lighter belly which is typical of the "agouti" pattern.

In addition to the coat color and pattern variations discussed above, many others have occurred in rodents which have been bred in the laboratory, though they are as yet known in too few species to make comparisons profitable. One of these, dilution, is a unit character in mice and rabbits. In this variation, the pigment granules are clumped and reduced in distribu-

tion, producing when acting on black varieties the familiar maltese color of blue rabbits and mice. The maltese cat is the result of a similar variation from black. Dilution is a simple Mendelian recessive to full color. The red and black blotching of guinea-pigs, the ticking or banding of the belly hairs in guinea-pigs, and the white bellies of a fancy variety of "agouti" mice are also known to be Mendelian unit characters.⁸

SUMMARY

A summary of the preceding discussion shows that we have examined five of the commonest variations in rodents: albinism, pink-eye, yellow, white-spotting, and black. In all of these the inheritance is known for at least three species, and in general the variant is recessive to the wild type. Dominant variations have occurred often enough (such as "English spotting" in rabbits, and black-eyed white-spotting and yellow in mice) to make us chary about drawing any general conclusions concerning the occurrence of evolution purely by loss mutations from type. The generality that does appear is rather the widespread occurrence in this order of similar variations both under domestication when the animals are saved and bred, and in the wild, when usually only the stuffed specimens are preserved. There is implied in this similarity, which in certain cases amounts to a homology, a similarity in that part of the organism which is responsible for the variations, i.e. the germ plasm. It may be that we know in these days as little concerning the causes of variation as did naturalists in the days of Lamarck or of Darwin. We do at least know where the

⁸ For a fuller discussion of these the interested reader is referred to Chapter XII in Castle's *Genetics and Eugenics*, 1920, and to the series of papers by Wright cited above.

causes are to be sought, and, once having arisen by a mysterious occurrence called mutation, we have learned something of the manner in which the variations are inherited, and by a process of inference have been able to localize still more exactly the region of change. The only permissible generality, then, concerns a general similarity in the germ plasm and probably in the individual chromosomes of these many species of rodents. But in one case the similarity between species has been found to be more than general. It has been found to be quite a specific similarity.

If we examine this case in detail we find that in two distinct, inter-sterile species, mice and rats, two similarly appearing variations have occurred, albinism and pink-eye. In rats the genes for these variations are linked with a strength of about 21 per cent, which is possibly slightly in excess of the actual. In mice the linkage between these genes is something less than 15 per cent, which is based on observations of 6700 animals raised solely for the purpose of determining this linkage and is probably reasonably accurate. In terms of the chromosome hypothesis these facts mean that these two genes are present in the same chromosome in rats and mice, in rats at a distance of 21 units apart, in mice at a distance of about 15 units apart. The difference in location is so small that for practical purposes we can say that they are located at homologous points in the two species.

In guinea-pigs where both of these variations occur, there is incomplete evidence, but the data which Doctor Wright has supplied indicate that pink-eye and albinism in guinea-pigs are probably not linked and may therefore be determined in different chromosomes. This does not prove, however, that these variations in guinea-

pigs are *not* the same as those in mice and rats. It may mean that the chromosome which contains both genes in the more primitive Muridæ may in the more specialized Caviidæ be represented by two chromosomes, the sum of which rather than either one separately may be homologous with the one chromosome of mice and rats. Although this will be recognized as speculation, there is some slight evidence that in the evolution of the rodents a fractionation of chromosomes may have occurred, for the mice and rats have 19 (haploid) while the guinea-pigs have 28. In the rabbit (*Oryctolagus cuniculus*) a member now judged too primitive for the true rodents and recently placed in the Lagomorpha with the others of the old rodent suborder Duplicidentata, the chromosome number is probably 12. If this progressive increase in the number of chromosomes through the order Rodentia is found to be a fact and not a chance phenomenon associated with the smallness of the sample of four species from which our cytological evidence is drawn, it may furnish a very important clue to a series of evolutionary relationships of more than ordinary interest.

In the concluding chapter of his recent book Professor Morgan (1919) has referred to the possible evolutionary significance of the localization of genes as determined by the study of linkage. He has there reviewed some of the work on similar variations in several species of insects by Metz and Sturtevant, pointing out the difficulties to be encountered in applying this method to the analysis of species, chief of which is the necessity of establishing the same linear order in each species of the genes for similar variations. A species in his point of view, and in this he follows De Vries, may ultimately prove to be a "community of

genes." We may expect evidence of this community in the variations which arise from time to time within the species, whether they be at the time of specific value or not. Such community is not to be inferred from mere similarity in appearance but must rest on a more real homology of germinal cause. This kind of similarity is now apparent between *Mus musculus* and *Rattus norvegicus*, which have varied so far from a common type that they are now inter-sterile and have been placed recently in different genera. Yet they have retained a genetic constitution so similar that it contains genes common to both species. Whether this is due to a community

of descent in the terms of current evolutionary theory or to relationship through some other cause is one of the questions which genetics, aided by the chromosome notation, may be expected at some time to answer.

BIBLIOGRAPHY

- Castle, W. E. 1920 *Genetics and Eugenics*. Harvard University Press, Cambridge, Mass.
- Morgan, T. H., Sturtevant, A. H., Muller, H. J., and Bridges, C. B. 1915 *The Mechanism of Mendelian Heredity*. Henry Holt and Co., New York.
- Morgan, T. H. 1919 *The Physical Basis of Heredity*. J. B. Lippincott Co., Philadelphia and London.
- Wright, S. 1917 "Color Inheritance in Mammals." *Journal of Heredity*, vol. 8, nos. 5-9.



Variation Due to Change in the Individual Gene

H. J. MULLER

Reprinted by author's and publisher's permission from *American Naturalist*, vol. 56, 1922, pp. 32-50.

It is not the practice of scientists to expend much effort in making their contributions readable. If a paper successfully communicates the experimental data and the conclusions drawn to the reader, the author is satisfied, and editors find such work quite acceptable. Once in a great while, however, science is blessed with an author both eminently successful at his trade and articulate, so that his contributions are a pleasure to read. Such a man is H. J. Muller. Students in my general biology course found this article the easiest of the entire collection to read and digest. In my opinion, it is the paper that taught them the most.

We have noted two different types of papers already; first, the research paper, and second, the paper embodying synthesis of several areas of study. Muller's contribution is a third type. It is a thoughtful analysis of the progress that had been made in the understanding of

a specific phenomenon, a summary of our knowledge concerning it, and then indication of the pathways that might prove fruitful for future research. This type of contribution is known as a "review paper," and, when properly done, serves as a primary tool used by biologists to keep up with progress in fields other than their own. Muller's paper not only provided this service for biologists of his time, but also stands as a valuable reference and a classic summary for anyone interested in the development of the theory of the gene. The validity of Muller's remarks concerning future research and its aims is highlighted by his paper published five years later, in which he announced successful experimental mutation (see p. 149).

I. THE RELATION BETWEEN THE GENES AND THE CHARACTERS OF THE ORGANISM

THE PRESENT PAPER WILL BE concerned rather with problems, and the possible means of attacking them, than with the details of cases and data. The opening up of these new problems is due to the fundamental contribution which genetics has made to cell physiology within the last decade. This contribution, which has so far scarcely been assimilated by the general physiologists themselves, consists in the demonstration that, besides the ordinary proteins, carbohydrates, lipoids, and extractives, of their several types, there are present within the cell *thousands* of distinct substances—the "genes"; these genes exist as ultra-microscopic particles; their influences nevertheless permeate the entire cell, and they play a fundamental rôle in determining the nature of all cell substances, cell structures, and cell activities. Through these cell effects, in turn, the genes affect the entire organism.

It is not mere guesswork to say that the genes are ultra-microscopic bodies. For the work on *Drosophila* has not only proved that the genes are in the chromosomes, in definite positions, but it has shown that there must be hundreds of such genes within each of the

larger chromosomes, although the length of these chromosomes is not over a few microns. If, then, we divide the size of the chromosome by the minimum number of its genes, we find that the latter are particles too small to give a visible image.

The chemical composition of the genes, and the formulae of their reactions, remain as yet quite unknown. We do know, for example, that in certain cases a given pair of genes will determine the existence of a particular enzyme (concerned in pigment production), that another pair of genes will determine whether or not a certain agglutinin shall exist in the blood, a third pair will determine whether homogentisic acid is secreted into the urine ("alkaptonuria"), and so forth. But it would be absurd, in the third case, to conclude that on this account the gene itself consists of homogentisic acid, or any related substance, and it would be similarly absurd, therefore, to regard cases of the former kind as giving any evidence that the gene is an enzyme, or an agglutinin-like body. The reactions whereby the genes produce their ultimate effects are too complex for such inferences. Each of these effects, which we call a "character" of the organism, is the product of a highly complex, intricate, and delicately balanced system of reactions, caused by the interaction of

countless genes, and every organic structure and activity is therefore liable to become increased, diminished, abolished, or altered in some other way, when the balance of the reaction system is disturbed by an alteration in the nature or the relative quantities of any of the component genes of the system. To return now to these genes themselves.

II. THE PROBLEM OF GENE MUTABILITY

The most distinctive characteristic of each of these ultra-microscopic particles—that characteristic whereby we identify it as a gene—is its property of self-propagation: the fact that, within the complicated environment of the cell protoplasm, it reacts in such a way as to convert some of the common surrounding material into an end-product identical in kind with the original gene itself. This action fulfills the chemist's definition of "autocatalysis"; it is what the physiologist would call "growth"; and when it passes through more than one generation it becomes "heredity." It may be observed that this reaction is in each instance a rather highly localized one, since the new material is laid down by the side of the original gene.

The fact that the genes have this autocatalytic power is in itself sufficiently striking, for they are undoubtedly complex substances, and it is difficult to understand by what strange coincidence of chemistry a gene can happen to have just that very special series of physico-chemical effects upon its surroundings which produces—of all possible end-products—just this particular one, which is identical with its own complex structure. But the most remarkable feature of the situation is not this oft-noted autocatalytic action in itself—it is the fact that, when the structure of the

gene becomes changed, through some "chance variation," the catalytic property of the gene may¹ become correspondingly changed, in such a way as to leave it still *autocatalytic*. In other words, the change in gene structure—accidental though it was—has somehow resulted in a change of exactly *appropriate* nature in the catalytic reactions, so that the new reactions are now accurately adapted to produce more material just like that in the new changed gene itself. It is this paradoxical phenomenon which is implied in the expression "variation due to change in the individual gene," or, as it is often called, "mutation."

What sort of structure must the gene possess to permit it to mutate in this way? Since, through change after change in the gene, this same phenomenon persists, it is evident that it must depend upon some general feature of gene construction—common to all genes—which gives each one a *general* autocatalytic power—a "*carte blanche*"—to build material of whatever specific sort it itself happens to be composed of. This general principle of gene structure might, on the one hand, mean nothing more than the possession by each gene of some very simple character, such as a particular radicle or "side-chain"—alike in them all—which enables each gene to enter into combination with certain highly organized materials in the outer protoplasm, in such a way as to result in the formation, "by" the protoplasm, of more material like this gene which is in combination with it. In that case the gene itself would only initiate and guide the direction of the reaction. On the other hand, the extreme alternative to such a conception has been gen-

¹ It is of course conceivable, and even unavoidable, that *some* types of changes do destroy the gene's autocatalytic power, and thus result in its eventual loss.

erally assumed, perhaps gratuitously, in nearly all previous theories concerning hereditary units; this postulates that the chief feature of the autocatalytic mechanism resides in the structure of the genes themselves, and that the outer protoplasm does little more than provide the building material. In either case, the question as to what the general principle of gene construction is, that permits this phenomenon of mutable autocatalysis, is the most fundamental question of genetics.

The subject of gene variation is an important one, however, not only on account of the apparent problem that is thus inherent in it, but also because this same peculiar phenomenon that it involves lies at the root of organic evolution, and hence of all the vital phenomena which have resulted from evolution. It is commonly said that evolution rests upon two foundations—inheritance and variation; but there is a subtle and important error here. Inheritance by itself leads to no change, and variation leads to no permanent change, unless the variations themselves are heritable. Thus it is not inheritance *and* variation which bring about evolution, but the inheritance of variation, and this in turn is due to the general principle of gene construction which causes the persistence of autocatalysis despite the alteration in structure of the gene itself. Given, now, any material or collection of materials having this one unusual characteristic, and evolution would automatically follow, for this material would, after a time, through the accumulation, competition and selective spreading of the self-propagated variations, come to differ from ordinary inorganic matter in innumerable respects, in addition to the original difference in its mode of catalysis. There would thus result a wide gap between

this matter and other matter, which would keep growing wider, with the increasing complexity, diversity and so-called "adaptation" of the selected mutable material.

III. A POSSIBLE ATTACK THROUGH CHROMOSOME BEHAVIOR

In thus recognizing the nature and the importance of the problem involved in gene mutability have we now entered into a *cul de sac*, or is there some way of proceeding further so as to get at the physical basis of this peculiar property of the gene? The problems of growth, variation and related processes seemed difficult enough to attack even when we thought of them as inherent in the organism as a whole or the cell as a whole—how now can we get at them when they have been driven back, to some extent at least, within the limits of an invisible particle? A gene can not effectively be ground in a mortar, or distilled in a retort, and although the physico-chemical investigation of other biological substances may conceivably help us, by analogy, to understand its structure, there seems at present no method of approach along this line.

There is, however, another possible method of approach available: that is, to study the behavior of the chromosomes, as influenced by their contained genes, in their various physical reactions of segregation, crossing over, division, synapsis, etc. This may at first sight seem very remote from the problem of getting at the structural principle that allows mutability in the gene, but I am inclined to think that such studies of synaptic attraction between chromosomes may be especially enlightening in this connection, because the most remarkable thing we know about genes—besides their mutable autocatalytic power—is the

highly specific attraction which like genes (or local products formed by them) show for each other. As in the case of the autocatalytic forces, so here the attractive forces of the gene are somehow exactly adjusted so as to react in relation to more material of the same complicated kind. Moreover, when the gene mutates, the forces become readjusted, so that they may now attract material of the new kind; this shows that the attractive or synaptic property of the gene, as well as its catalytic property, is not primarily dependent on its specific structure, but on some general principle of its make-up, that causes whatever specific structure it has to be auto-attractive (and autocatalytic).

This auto-attraction is evidently a strong force, exerting an appreciable effect against the non-specific mutual repulsions of the chromosomes, over measurable microscopic distances much larger than in the case of the ordinary forces of so-called cohesion, adhesion and adsorption known to physical science. In this sense, then, the physicist has no parallel for this force. There seems, however, to be no way of escaping the conclusion that in the last analysis it must be of the same nature as these other forces which cause inorganic substances to have specific attractions for each other, according to their chemical composition. These inorganic forces, according to the newer physics, depend upon the arrangement and mode of motion of the electrons constituting the molecules, which set up electro-magnetic fields of force of specific patterns. To find the principle peculiar to the construction of the force-field pattern of genes would accordingly be requisite for solving the problem of their tremendous auto-attraction.

Now, according to Troland (1917), the growth of crystals from a solution

is due to an attraction between the solid crystal and the molecules in solution caused by the similarity of their force field patterns, somewhat as similarly shaped magnets might attract each other—north to south poles—and Troland maintains that essentially the same mechanism must operate in the autocatalysis of the hereditary particles. If he is right, each different portion of the gene structure must—like a crystal—attract to itself from the protoplasm materials of a similar kind, thus moulding next to the original gene another structure with similar parts, identically arranged, which then become bound together to form another gene, a replica of the first. This does not solve the question of what the general principle of gene construction is, which permits it to retain, like a crystal, these properties of auto-attraction,² but if the main point is correct, that the autocatalysis is an expression of specific attractions between portions of the gene and similar protoplasmic building blocks (dependent on their force-field patterns), it is evident that the very same forces which cause the genes to grow should also cause like genes to attract each other, but much more strongly, since here all the individual attractive forces of the different parts of the gene are summated. If the two phenomena are thus really dependent on a common principle in the make-up of the gene,

² It can hardly be true, as Troland intimates, that all similar fields attract each other more than they do dissimilar fields, otherwise all substances would be autocatalytic, and, in fact, no substances would be soluble. Moreover, if the parts of a molecule are in any kind of "solid," three dimensional formation, it would seem that those in the middle would scarcely have opportunity to exert the moulding effect above mentioned. It therefore appears that a special manner of construction must be necessary, in order that a complicated structure like a gene may exert such an effect.

progress made in the study of one of them should help in the solution of the other.

Great opportunities are now open for the study of the nature of the synaptic attraction, especially through the discovery of various races having abnormal numbers of chromosomes. Here we have already the finding by Belling, that where three like chromosomes are present, the close union of any two tends to exclude their close union with the third. This is very suggestive, because the same thing is found in the cases of specific attractions between inorganic particles, that are due to their force field patterns. And through Bridges' finding of triploid *Drosophila*, the attraction phenomena can now be brought down to a definitely genic basis, by the introduction of specific genes—especially those known to influence chromosome behavior—into one of the chromosomes of a triad. The amount of influence of this gene on attraction may then be tested quantitatively, by genetic determination of the frequencies of the various possible types of segregation. By extending such studies to include the effect of various conditions of the environment—such as temperature, electrostatic stresses, etc.—in the presence of the different genetic situations, a considerable field is opened up.

This suggested connection between chromosome behavior and gene structure is as yet, however, only a possibility. It must not be forgotten that at present we can not be sure that the synaptic attraction is exerted by the genes themselves rather than by local products of them, and it is also problematical whether the chief part of the mechanism of autocatalysis resides within the genes rather than in the "protoplasm." Meanwhile, the method is worth following up, simply because

it is one of our few conceivable modes of approach to an all-important problem.

It may also be recalled in this connection that besides the genes in the chromosomes there is at least one similarly autocatalytic material in the chloroplastids, which likewise may become permanently changed, or else lost, as has been shown by various studies on chlorophyll inheritance. Whether this plastid substance is similar to the genes in the chromosomes we can not say, but of course it can not be seen to show synaptic attraction, and could not be studied by the method suggested above.³

IV. THE ATTACK THROUGH STUDIES OF MUTATION

There is, however, another method of attack, in a sense more direct, and not open to the above criticisms. That is the method of investigating the individual gene, and the structure that permits it to change, through a study of the changes themselves that occur in it, as observed by the test of breeding and development. It was through the investigation of the *changes* in the chromosomes—caused by crossing over—that the structure of the chromosomes was analyzed into their constituent genes in line formation; it was through study of molecular changes that molecules were analyzed into atoms tied together in definite ways, and it has been finally the rather recent finding of changes in atoms and investigation of the resulting pieces, that has led us to the present analysis of atomic structure into positive and negative electrons having characteristic arrangements. Similarly, to under-

³ It may be that there are still other elements in the cell which have the nature of genes, but as no critical evidence has ever been adduced for their existence, it would be highly hazardous to postulate them.

stand the properties and possibilities of the individual gene, we must study the mutations as directly as possible, and bring the results to bear upon our problem.

(a) *The Quality and Quantity of the Change*

In spite of the fact that the drawing of inferences concerning the gene is very much hindered, in this method, on account of the remoteness of the gene-cause from its character-effect, one salient point stands out already. It is that the change is not always a mere loss of material, because clear-cut reverse mutations have been obtained in corn, *Drosophila*, *Portulaca*, and probably elsewhere. If the original mutation was a loss, the reverse must be a gain. Secondly, the mutations in many cases seem not to be quantitative at all, since the different allelomorphs formed by mutations of one original gene often fail to form a single linear series. One case, in fact, is known in which the allelomorphs even affect totally different characters: this is the case of the truncate series, in which I have found that different mutant genes at the same locus may cause either a shortening of the wing, an eruption on the thorax, a lethal effect, or any combination of two or three of these characters. In such a case we may be dealing either with changes of different types occurring in the same material or with changes (possibly quantitative changes, similar in type) occurring in different component parts of one gene. Owing to the universal applicability of the latter interpretation, even where allelomorphs do not form a linear series, it can not be categorically denied, in any individual case, that the changes may be merely quantitative changes of some part of the gene. If all changes were thus quantitative, even in this limited sense

of a loss or gain of part of the gene, our problem of why the changed gene still seems to be autocatalytic would in the main disappear, but such a situation is excluded a priori since in that case the thousands of genes now existing could never have evolved.

Although a given gene may thus change in various ways, it is important to note that there is a strong tendency for any given gene to have its changes of a particular kind, and to mutate in one direction rather than in another. And although mutation certainly does not always consist of loss, it often gives effects that might be termed losses. In the case of the mutant genes for bent and eyeless in the fourth chromosome of *Drosophila* it has even been proved, by Bridges, that the effects are of exactly the same kind, although of lesser intensity, as those produced by the entire loss of the chromosome in which they lie, for flies having bent or eyeless in one chromosome and lacking the homologous chromosome are even more bent, or more eyeless, than those having a homologous chromosome that also contains the gene in question. The fact that mutations are usually recessive might be taken as pointing in the same direction, since it has been found in several cases that the loss of genes—as evidenced by the absence of an entire chromosome of one pair—tends to be much more nearly recessive than dominant in its effect.

The effect of mutations in causing a loss in the characters of the organism should, however, be sharply distinguished from the question of whether the gene has undergone any loss. It is generally true that mutations are much more apt to cause an apparent loss in character than a gain, but the obvious explanation for that is, not because the gene tends to lose something, but because most characters require for

proper development a nicely adjusted train of processes, and so any change in the genes—no matter whether loss, gain, substitution or rearrangement—is more likely to throw the developmental mechanism out of gear, and give a “weaker” result, than to intensify it. For this reason, too, the most frequent kind of mutation of all is the lethal, which leads to the loss of the entire organism, but we do not conclude from this that all the genes had been lost at the time of the mutation. The explanation for this tendency for most changes to be degenerative, and also for the fact that certain other kinds of changes—like that from red to pink eye in *Drosophila*—are more frequent than others—such as red to brown or green eye—lies rather in developmental mechanics than in genetics. It is because the developmental processes are more unstable in one direction than another, and easier to push “downhill” than up, and so any mutations that occur—no matter what the gene change is like—are more apt to have these effects than the other effects. If now selection is removed in regard to any particular character, these character changes which occur more readily must accumulate, giving apparent orthogenesis, disappearance of unused organs, of unused physiological capabilities, and so forth. As we shall see later, however, the changes are not so frequent or numerous that they could ordinarily push evolution in such a direction against selection and against the immediate interests of the organism.

In regard to the magnitude of the somatic effect produced by the gene variation, the *Drosophila* results show that there the smaller character changes occur oftener than large ones. The reason for this is again probably to be found in developmental mechanics, owing to the fact that there

are usually more genes slightly affecting a given character than those playing an essential rôle in its formation. The evidence proves that there are still more genes whose change does not affect the given character at all—no matter what this character may be, unless it is life itself—and this raises the question as to how many mutations are absolutely unnoticed, affecting no character, or no detectable character, to any appreciable extent at all. Certainly there must be many such mutations, judging by the frequency with which “modifying factors” arise, which produce an effect only in the presence of a special genetic complex not ordinarily present.

(b) The Localization of the Change

Certain evidence concerning the causation of mutations has also been obtained by studying the relations of their occurrence to one another. Hitherto it has nearly always been found that only one mutation has occurred at a time, restricted to a single gene in the cell. I must omit from consideration here the two interesting cases of deficiency, found by Bridges and by Mohr, in each of which it seems certain that an entire region of a chromosome, with its whole cargo of genes, changed or was lost, and also a certain peculiar case, not yet cleared up, which has recently been reported by Nilsson-Ehle; these important cases stand alone. Aside from them, there are only two instances in which two (or more) new mutant genes have been proved to have been present in the same gamete. Both of these are cases in *Drosophila*—reported by Muller and Altenburg (1921)—in which a gamete contained two new sex-linked lethals; two cases are not a greater number than was to have been expected from a random distri-

bution of mutations, judging by the frequency with which single mutant lethals were found in the same experiments. Ordinarily, then, the event that causes the mutation is specific, affecting just one particular kind of gene of all the thousands present in the cell. That this specificity is due to a spatial limitation rather than a chemical one is shown by the fact that when the single gene changes the other one, of identical composition, located near by in the homologous chromosome of the same cell, remains unaffected. This has been proved by Emerson in corn, by Blakeslee in *Portulaca*, and I have shown there is strong evidence for it in *Drosophila*. Hence these mutations are not caused by some general pervasive influence, but are due to "accidents" occurring on a molecular scale. When the molecular or atomic motions chance to take a particular form, to which the gene is vulnerable, then the mutation occurs.

It will even be possible to determine whether the entire gene changes at once, or whether the gene consists of several molecules or particles, one of which may change at a time. This point can be settled in organisms having determinate cleavage, by studies of the distribution of the mutant character in somatically mosaic mutants. If there is a group of particles in the gene, then when one particle changes it will be distributed irregularly among the descendant cells, owing to the random orientation of the two halves of the chromosome on the mitotic spindles of succeeding divisions,⁴ but if there is only one particle

to change, its mutation must affect all of the cells in a *bloc*, that are descended from the mutant cell.

(c) The Conditions under which the Change occurs

But the method that appears to have most scope and promise is the experimental one of investigating the conditions under which mutations occur. This requires studies of mutation frequency under various methods of handling the organisms. As yet, extremely little has been done along this line. That is because, in the past, a mutation was considered a windfall, and the expression "mutation frequency" would have seemed a contradiction in terms. To attempt to study it would have seemed as absurd as to study the conditions affecting the distribution of dollar bills on the sidewalk. You were simply fortunate if you found one. Not even controls, giving the "normal" rate of mutation—if indeed there is such a thing—were attempted.⁵ Of late, however, we may say that certain very exceptional banking houses have been found, in front of which the dollars fall more frequently—in other words, specially mutable genes have been discovered, that are beginning to yield abundant data at the hands of Nilsson-Ehle, Zeleny, Emerson, Anderson and others. For some of these mutable genes the rate of change is found to be so rapid that at the end of a few decades half of

tend not to persist as such, for the occurrence of mutation in one particle after the other would in time differentiate the gene into a number of different genes consisting of one particle each.

⁴ This depends on the assumption that if the gene does consist of several particles, the halves of the chromosomes, at each division, receive a random sample of these particles. That is almost a necessary assumption, since a gene formed of particles each one of which was separately partitioned at division would

⁵ Studies of "mutation frequency" had of course been made in the *CEnotheras*, but as we now know that these were not studies of the rate of gene change but of the frequencies of crossing over and of chromosome aberrations they may be neglected for our present purposes.

the genes descended from those originally present would have become changed. After these genes have once mutated, however, their previous mutability no longer holds. In addition to this "banking house method" there are also methods, employed by Altenburg and myself, for—as it were—automatically sweeping up wide areas of the streets and sifting the collections for the valuables. By these special genetic methods of reaping mutations we have recently shown that the ordinary genes of *Drosophila*—unlike the mutable genes above—would usually require at least a thousand years—probably very much more—before half of them became changed. This puts their stability about on a par with, if not much higher than, that of atoms of radium—to use a fairly familiar analogy. Since, even in these latter experiments, many of the mutations probably occurred within a relatively few rather highly mutable genes, it is likely that most of the genes have a stability far higher than this result suggests.

The above mutation rates are mere first gleanings—we have yet to find how different conditions affect the occurrence of mutations. There had so far been only the negative findings that mutation is not confined to one sex (Muller and Altenburg, 1919; Zeleny, 1921), or to any one stage in the life cycle (Bridges, 1919; Muller, 1920; Zeleny, 1921), Zeleny's finding that bar-mutation is not influenced by recency of origin of the gene (1921), and the as yet inconclusive differences found by Altenburg and myself for mutation rate at different temperatures (1919), until at this year's meeting of the botanists Emerson announced the definite discovery of the influence of a genetic factor in corn upon the mutation rate in its allelomorph, and Anderson the finding of

an influence upon mutation in this same gene, caused by developmental conditions—the mutations from white to red of the mutable gene studied occurring far more frequently in the cells of the more mature ear than in those of the younger ear. These two results at least tell us decisively that mutation is not a sacred, inviolable, unapproachable process: it may be altered. These are the first steps; the way now lies open broad for exploration.

It is true that I have left out of account here the reported findings by several investigators, of genetic variations caused by treatments with various toxic substances and with certain other unusual conditions. In most of these cases, however, the claim has not been made that actual gene changes have been caused: the results have usually not been analyzed genetically and were in fact not analyzable genetically; they could just as well be interpreted to be due to abnormalities in the distribution of genes—for instance, chromosome abnormalities like those which Mavor has recently produced with X-rays—as to be due to actual gene mutations. But even if they were due to real genic differences, the possibility has in most cases by no means been excluded (1) that these genic differences were present in the stock to begin with, and merely became sorted out unequally, through random segregation; or (2) that other, invisible genic differences were present which, after random sorting out, themselves caused differences in mutation rate between the different lines. Certain recent results by Altenburg and myself suggest that genic differences, affecting mutation rate, may be not uncommon. To guard against either of these possibilities it would have been necessary to test the stocks out by a thorough course of inbreed-

ing beforehand, or else to have run at least half a dozen different pairs of parallel lines of the control and treated series, and to have obtained a definite difference in the same direction between the two lines of *each* pair; otherwise it can be proved by the theory of "probable error" that the differences observed may have been a mere matter of random sampling among genic differences originally present. Accumulating large numbers of abnormal or inferior individuals by selective propagation of one or two of the treated lines—as has been done in some cases—adds nothing to the significance of the results.

At best, however, these genetically unrefined methods would be quite insensitive to mutations occurring at anything like ordinary frequency, or to such differences in mutation rate as have already been found in the analytical experiments on mutation frequency. And it seems quite possible that larger differences than these will not easily be hit upon, at least not in the early stages of our investigations, in view of the evidence that mutation is ordinarily due to an accident on an ultramicroscopic scale, rather than directly caused by influences pervading the organism. For the present, then, it appears most promising to employ organisms in which the genetic composition can be controlled and analyzed, and to use genetic methods that are sensitive enough to disclose mutations occurring in the control as well as in the treated individuals. In this way relatively slight variations in mutation frequency, caused by the special treatments, can be determined, and from the conditions found to alter the mutation rate slightly we might finally work up to those which affect it most markedly. The only methods now meeting this requirement are those in which a particular mutable gene is fol-

lowed, and those in which many homozygous or else genetically controlled lines can be run in parallel, either by parthenogenesis, self-fertilization, balanced lethals or other special genetic means, and later analyzed, through sexual reproduction, segregation and crossing over.

V. OTHER POSSIBILITIES

We can not, however, set fixed limits to the possibilities of research. We should not wish to deny that some new and unusual method may at any time be found of directly producing mutations. For example, the phenomena now being worked out by Guyer may be a case in point. There is a curious analogy between the reactions of immunity and the phenomena of heredity, in apparently fundamental respects,⁶ and any results that seem to connect the two are worth following to the limit.

Finally, there is a phenomenon related to immunity, of still more striking nature, which must not be neglected by geneticists. This is the d'Hérelle phenomenon. D'Hérelle found in 1917 that the presence of dysentery bacilli in the body caused the production there of a filterable substance, emitted in the stools, which had a lethal and in fact dissolving action on the corresponding type of bacteria, if a drop of it were applied to

⁶ I refer here to the remarkable specificity with which a particular complex antigen calls forth processes that construct for it an antibody that is attracted to it and fits it "like lock and key," followed by further processes that cause more and more of the antibody to be reproduced. If the antigen were a gene, which could be slightly altered by the cell to form the antibody that neutralized it—as some enzymes can be slightly changed by heating so that they counteract the previous active enzyme—and if this antibody-gene then became implanted in the cell so as to keep on growing, all the phenomena of immunity would be produced.

a colony of the bacteria that were under cultivation. So far, there would be nothing to distinguish this phenomenon from immunity. But he further found that when a drop of the affected colony was applied to a second living colony, the second colony would be killed; a drop from the second would kill a third colony, and so on indefinitely. In other words, the substance, when applied to colonies of bacteria, became multiplied or increased, and could be so increased indefinitely; it was self-propagable. It fulfills, then, the definition of an autocatalytic substance, and although it may really be of very different composition and work by a totally different mechanism from the genes in the chromosomes, it also fulfills our definition of a gene.⁷ But the resemblance goes further—it has been found by Gratia that the substance may, through appropriate treatments on other bacteria, become changed (so as to produce a somewhat different effect than before, and attack different bacteria) and still retain its self-propagable nature.

That two distinct kinds of substances—the d'Hérelle substances and the genes—should both possess this most remarkable property of heritable variation or “mutability,” each working by a totally different mechanism, is quite conceivable, considering the complexity of protoplasm, yet it would seem a curious coincidence indeed. It would open up the possibility of two totally different kinds of life, working by different mechanisms. On the other hand, if these d'Hérelle bodies were really genes, fundamen-

tally like our chromosome genes, they would give us an utterly new angle from which to attack the gene problem. They are filterable, to some extent isolable, can be handled in test-tubes, and their properties, as shown by their effects on the bacteria, can then be studied after treatment. It would be very rash to call these bodies genes, and yet at present we must confess that there is no distinction known between the genes and them. Hence we can not categorically deny that perhaps we may be able to grind genes in a mortar and cook them in a beaker after all. Must we geneticists become bacteriologists, physiological chemists and physicists, simultaneously with being zoologists and botanists? Let us hope so.

I have purposely tried to paint things in the rosiest possible colors. Actually, the work on the individual gene, and its mutation, is beset with tremendous difficulty. Such progress in it as has been made has been by minute steps and at the cost of infinite labor. Where results are thus meager, all thinking becomes almost equivalent to speculation. But we can not give up thinking on that account, and thereby give up the intellectual incentive to our work. In fact, a wide, unhampered treatment of all possibilities is, in such cases, all the more imperative, in order that we may direct these labors of ours where they have most chance to count. We must provide eyes for action.

The real trouble comes when speculation masquerades as empirical fact. For those who cry out most loudly against “theories” and “hypotheses”—whether these latter be the chromosome theory, the factorial “hypothesis,” the theory of crossing over, or any other—are often the very ones most guilty of stating their results in terms that make illegitimate *implicit*

⁷ D'Hérelle himself thought that the substance was a filterable virus parasitic on the bacterium, called forth by the host body. It has since been found that various bacteria each cause the production of d'Hérelle substances which are to some extent specific for the respective bacteria.

assumptions, which they themselves are scarcely aware of simply because they are opposed to dragging "speculation" into the open. Thus they may be finally led into the worst blunders of all. Let us, then, frankly admit the uncertainty of many of the possibilities we have dealt with, using them as a spur to the real work.

LITERATURE CITED

- Blakeslee, A. F. 1920 A Dwarf Mutation in *Portulaca* showing Vegetative Reversions. *Genetics*, vol. 5, pp. 419-433.
- Bridges, C. B. 1917 Deficiency. *Genetics*, vol. 2, pp. 445-465.
- _____, 1919 The Developmental Stages at which Mutations Occur in the Germ Tract. *Proc. Soc. Exp. Biol. and Med.*, vol. 17, pp. 1-2.
- _____, 1921 Genetical and Cytological Proof of Non-disjunction of the Fourth Chromosome of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.*, vol. 7, pp. 186-192.
- D'Hérelle, F. 1917 *Compt. rend. Acad.*, vol. 165, p. 373.
- 1918 *Compt. rend. Acad.*, vol. 167, p. 970.
- 1918 *Compt. rend. Soc. Biol.*, vol. 81, p. 1160.
- 1919 *Compt. rend. Acad.*, vol. 168, p. 631.
- 1920 *Compt. rend. Soc. Biol.*, vol. 83, pp. 52, 97, 247.
- Emerson, R. A. 1911 The Inheritance of a Recurring Somatic Variation in Variegated Ears of Maize. *Amer. Nat.*, vol. 48, pp. 87-115.
- Gratia, A. 1921 Studies on the d'Hérelle Phenomenon. *Jour. Exp. Med.*, vol. 34, pp. 115-126.
- Mavor, J. W. 1921 On the Elimination of the X-chromosome from the egg of *Drosophila melanogaster* by X-rays. *Science*, n.s., 54, pp. 277-279.
- Mohr, O. L. 1919 Character Changes caused by Mutation of an Entire Region of a Chromosome in *Drosophila*. *Genetics*, vol. 4, pp. 275-282.
- Muller, H. J. 1920 Further Changes in the White-eye Series of *Drosophila* and their Bearing on the Manner of Occurrence of Mutation. *Jour. Exp. Zool.*, vol. 31, pp. 443-473.
- Muller, H. J., and E. Altenburg, 1919 The Rate of Change of Hereditary Factors in *Drosophila*. *Proc. Soc. Exp. Biol. and Med.*, vol. 17, pp. 10-14.
- _____, 1921 A Study of the Character and Mode of Origin of 18 Mutations in the X-chromosome of *Drosophila*. *Anat. Rec.*, vol. 20, p. 213.
- Nilsson-Ehle, H. 1911 Ueber Fälle spontanen Wegfallens eines Hemmungsfaktors beim Hafer. *Zeit. f. Ind. Abst. u. Vererb.*, vol. 5, pp. 1-37.
- _____, 1920 Multiple Allelomorphe und Komplexmutationen beim Weizen. *Hereditas*, vol. 1, pp. 277-312.
- Troland, L. T. 1917 Biological Enigmas and the Theory of Enzyme Action. *Amer. Nat.*, vol. 51, pp. 321-350.
- Wollstein, M. 1921 Studies on the Phenomenon of d'Hérelle with *Bacillus dysenteriae*. *Jour. Exp. Med.*, vol. 34, pp. 467-477.
- Zeleny, C. 1920 The Direction and Frequency of Mutation in a Series of Multiple Allelomorphs. *Anat. Rec.*, vol. 20, p. 210.
- _____, 1921 The Direction and Frequency of Mutation in the Bar-eye Series of Multiple Allelomorphs of *Drosophila*. *Jour. Exp. Zool.*, vol. 34, pp. 203-233.



Sex in Relation to Chromosomes and Genes

CALVIN B. BRIDGES

Reprinted by publisher's permission from *American Naturalist*, vol. 59, 1925, pp. 127-137.

It sometimes happens in biology that a hypothesis that fits all known facts and evidence is offered and accepted for many years. It may prove to be so completely satisfactory that it becomes accepted as fact rather than as hypothesis. This was true of sex determination, which was long understood to be a simple matter of the presence or absence of a particular chromosome. In mammals and other groups it was a "Y" chromosome, which, present and paired with an "X" chromosome, gave a male. If the Y was absent, and two X chromosomes were paired, a female was born. The presence and absence theory was sufficient to explain sex determination in many different kinds of plants and animals. Only the occurrence of a situation the concept could not explain led Bridges to formulate a different theory—that of chromosome balance.

We still do not know today how widely the balance theory of sex determination can be applied. Bridges shows in this paper that it might apply in the case of several plant species, but this is primarily on the strength of extrapolated evidence. But many geneticists today believe that the hypothesis of the balanced chromosome determination of sex in the fruit fly is equally valid throughout a wide range of species, and this paper stands as a classic in the study of sex determination.

DURING THE THREE YEARS SINCE the report at the Toronto meeting (Bridges '22) considerable new information has accumulated with regard to the series of different sex-types that has arisen in the breeding work with *Drosophila* (Table I). Each of these different sex-types is the result of a particular combination of chromosomes. They occur principally among the offspring of females that are triploids, that is, that have three

X-chromosomes and three of each kind of autosome. The possession of an extra X and at the same time of an extra set of autosomes does not change this individual in its sexual characters from the normal type of female. However, in gametogenesis the 3N group is an unstable one. Each egg receives a full set of chromosomes and a full set goes to the polar body. The members of the extra set are distributed between the egg and the polar body

in all possible combinations. Thus, a common type of egg of a 3N female has an extra set of autosomes. If we represent a set of autosomes by A, this egg can be formulated as X+A+A or X,2A. When such an egg is fertilized by an ordinary sperm, that can be formulated X+A, the zygote is 2X,-3A. This type of zygote develops into

an intersex, that is, into an individual that is neither male or female but an intermediate, or rather a mixture of male and female parts, very similar in type to the intersexes that Goldschmidt has worked with so extensively in *Lymantria dispar* (Goldschmidt '20).

The interpretation of these inter-

TABLE I
Relation of Sex to Chromosomes in *Drosophila melanogaster*

Sex Type	X (100)	A (80)	Sex Index	Interval	X = -6 A = +2
Superfemale	3	2	1.88	50%	-14
4N	4	4	1.25	—	-20
Female { 3N	3	3	1.25	—	-12
2N	2	2	1.25	—	-8
N*	1	1	1.25	50%	-4
Intersex { ♀ type	2	3	.83	—	-6
♂ type	2	3-IV	.83—	33%	-6
Male	1	2	.63	50%	-2
Supermale	1	3	.42	—	0

* The haploid type has not been discovered.

sexes in terms of genes carried in the chromosomes was made possible as the result of the very extensive studies of the manner in which ordinary characters are determined, and especially from the study of the contrasted character changes brought about on the one hand by the loss, and on the other hand by the gain, of one of the small round chromosomes, the fourth chromosome. From such studies the view had been reached that each character of an individual is the index of the point of balance in effectiveness of a large but unknown number of genes, some of which have a tendency to change development in one direction and others in the opposite (Bridges '22). This conception of "genic balance" was applied to the sex characters of the intersexes as follows: In chromosome constitution the intersexes differ from females only in that they

have an extra set of autosomes. This proves that the autosomes are concerned with the determination of sex. Moreover, they are male-determining in their action, since the addition of a set of autosomes causes the female to assume male characteristics. That is to say, in the autosomes there are genes that tend to produce the characters we call male, and these are more effective, either through greater numbers or through greater potency, than the total of autosomal genes tending to produce the alternative characters that we call female. On the other hand, the X has a net female tendency, as shown by the fact that the addition of an X to a male group changes the individual into a female. The net male tendency of a set of autosomes is less than the net female tendency of an X. This is seen in the fact that in the individual in which there are two of each,

namely 2X,2A, the female genes outweigh the male and the result is a female. If we represent the net effectiveness of the female tendency genes in the X by 100, then we should represent the net male effectiveness of a set of autosomes by some lower number; let us say 80. In a 2X,2A individual the ratio of female effectiveness to male effectiveness is 200:160, or 1.25 to 1; and on this formulation the sex index of 1.25 corresponds to the normal female. In the X,2A individual the ratio of female to male effectiveness is 100:160; or the sex index of a normal male is 0.63. In the 2X,3A intersex the ratio is 200:240, and the sex index is 0.83, which is intermediate between the indices for female and male. In the 3N female the ratio is 300:240, and the sex index is 1.25, exactly the same as in the normal female. This identity of sex indices for the 3N and 2N forms corresponds to the observation that there seems to be no strictly sexual differences between them. The larger size, coarser texture of eye, etc., of the 3N can be directly attributed to the changed volume of the nucleus, and are not sexual in nature.

Another type of egg of the 3N ♀ is X+X+A; and this, fertilized by a normal XA sperm, gives a 3X,2A individual with a sex index of 1.88, which is 50 per cent higher than that of the normal female. This constitution corresponds in fact to the "superfemales" that occur in these cultures and elsewhere. The superfemales are much delayed in development, are rarely able to live and are probably completely sterile.

Conversely, an X+A+A egg, fertilized by the type of sperm that does not carry an X, gives an X,3A zygote with a sex-index of only 0.42. This type of individuals was expected to be more male-like than an ordinary male; and such individuals were looked for

among the offspring of 3N females. At first none were found; but presently it was discovered that very late in the cultures an occasional example of a distinct type of male occurred. These so-called "supermales" are likewise sterile. Recently, cytological proof has been secured that this type of sex has the constitution X,3A, which agrees with the genetical evidence previously secured.

It was observed that the intersexes showed considerable variation and seemed to form a bimodal class. And since the cytological investigation had showed that some intersexes had three and others only two of the small round fourth chromosome, it was guessed that the more male-like mode corresponded to the full trio of fourth chromosomes, while the more female-like mode corresponded to the cytological type that lacked one fourth chromosome. An effort has been made to secure cytological evidence on this point. But this evidence is inconclusive; as is also that from an attempt to make a genetic test of the number of fourth chromosomes present through use of the fourth-chromosome mutant character eyeless. At present extra fourth chromosomes are being artificially inserted into the intersexes by continually crossing 3N mothers to males known to have an extra fourth chromosome. Contrariwise, in other lines of intersexes, fourth chromosomes are being diminished in numbers by continually mating 3N mothers to males known to lack one of the two fourth chromosomes. Similarly, the supermaleness of the 3X,2A individuals might be reduced or be increased by matings with triplo-IV males or with haplo-IV males. For this experiment females are being used whose two X-chromosomes are permanently attached to each other (L. V. Morgan '22), and hence that give through non-

disjunction a very high proportion of 3X-superfemales. Present indications, from the uncompleted experiments, are slightly contradictory, but tend to a conclusion which is the opposite of that earlier reported as probable on the basis of the slight evidence then available (Bridges '22). When the number of fourth chromosomes is three the intersexes are more female-like, and when the number is two they are more male-like. The fourth chromosome has a net female tendency, similar to that of the X and different from that of the other autosomes. By variation in the number of fourth chromosomes it is possible to have a fringe of minor sex-types about each of the major types of sex difference.

The list of sex-types has been enlarged by the discovery of tetraploids, or 4N individuals. These are females, quite identical with normal females in sex characteristics. The tetraploid arose in a stock of triploids; and was detected only by the strikingly different offspring given. A female supposed to be 3N was selected from the 3N stock and outcrossed to a normal male. All the offspring were triploid females (about 30) or triploid intersexes (about 20). There were no 2N offspring or supersexes. It was seen that this result might be produced if the mother were 4N instead of 3N. For in that case all the reduced eggs would be 2N; and these fertilized by X-sperm would give 3N females, and fertilized by Y-sperm would give 2X,3A intersexes.

Before the discovery of this 4N individual, an expectation that it would occur had arisen from several facts. Thus, in the three years following the discovery of triploidy there had been found no less than twenty-five instances of the new occurrence of triploidy. This very high frequency was paralleled by cytological observations

that give the explanation of the origin of triploids. In three separate preparations of ordinary 2N females it was found that a portion of an ovary was constituted of markedly larger cells; and in two of the individuals some of the giant cells were in division, and the chromosomes could be counted as 4N. Evidently there had been in some oogonial cell a division of the chromosomes that had not been followed by division of the nucleus and cytoplasm. The resulting tissue was tetraploid, and any reduced gamete would be 2N. Such a 2N gamete, fertilized by a normal sperm, would account for each of the twenty-five recurrences of triploidy.

Furthermore, in examining sections of intersexes, two individuals were found in which similar cysts of even larger cells were present. In one of these cysts divisions were occurring; and the chromosomes were clearly 6N. A 6N cyst in a 3N female would give, upon reduction, 3N eggs, which, fertilized by X sperm, would give the expected 4N type of female.

Soon after this first case of 4N female a second similar case was found. Also L. V. Morgan found a third case, and was able to prove by genetic tests that four separate X-chromosomes had been present (in press).

The fact that 4N individuals are females, not modified as to sex, has important bearings on our ideas as to the way in which genes interact to produce their effect. The view adopted here is that in general the effectiveness is in proportion to number of genes, and the significant point is the *ratio* between sets of genes that tend to produce alternative effects. On this view we find a ready explanation of the fact that such diverse forms as 2N, 3N and 4N individuals are precisely alike in their sexual characteristics; for in all these forms the effec-

tiveness of both contending sets of influences has been doubled, trebled or quadrupled; and the ratio remains constant.

But a system of formulation different from the ratio type has been adopted by Goldschmidt in dealing with the intersexes produced in the course of his brilliant work on racial crosses of *Lymantria dispar*. To the male tendency of a particular race he assigns a positive value that is proportional to the strength of the male-determining gene or genes. To the female tendency he assigns another value also proportionate to the strength of the female-determining gene or genes. He then assumes that when in an individual the male value is greater than the female value the individual is a male, and that, conversely, when the female value is greater than the male by this same number of units the individual is a female. The locus of the male tendency gene (M) is in the "Z-chromosome" of which two are present in the male and one in the female. The female tendency is strictly maternally inherited; and hence the locus of the F genes is in the W-chromosome that descends from mother to daughter. The F gene is supposed to exert its influence on the cytoplasm of the developing egg; and hence, although the male has no W-chromosome, he is supposed to have a definite female tendency that was impressed upon the cytoplasm of the egg and that persists throughout development. For a "weak" race the value assigned to F is 80, and to M, 60. In the WZ individual the cytoplasmic [F] of 80 exceeds the M value of 60 by "the epistatic minimum" of 20 units, and the individual is a female. Likewise, in the ZZ individual the [F] is 80, but the net M value is twice 60 or 120, with an excess in the male direction of

40 units. For a strong race both F and M are higher, for example, 100 and 80, but the arithmetical relation between the values of F and M would still govern the sex of the individual. In a cross between a weak female and a strong male the ZW individual received an [F] of 80 from the mother and an M of 80 from the father. The values are thus balanced midway between the excesses necessary for a female on the one hand or a male on the other, and the result is an intersex. This far the formulation is satisfactory; but when extensive series of crosses are compared, and an attempt is made to give values to the F and the M of each race that will hold throughout the entire range of experiments, this attempt is rather unsuccessful. It seems to me that a reformulation on the ratio instead of the algebraic basis would give a series of consistent indices without running counter to the very valuable physiological ideas that Goldschmidt has developed.

In the table of sex-types of *Drosophila* the haploid individual is entered with the index 1.25, the same as that of the 2N, 3N and 4N females. Unfortunately the haploid individual has *not* been discovered. But it is plain from the view just given that the expectation for a haploid *Drosophila melanogaster* is that it would be female in sex character. Accordingly, it is necessary to assume that the sex-determining mechanism here is essentially different from that in the bee and similar forms in which the haploid individual is a male. To me, sex-determination in the bee is the outstanding unsolved puzzle, although before the development of the idea of genic balance it seemed one of the clearest and simplest of cases. If it is true that the male is a haploid individual, then one would suppose that the diploid in-

dividual should likewise be a male, since the ratios among the sex-determining genes are not different in the two cases.

Schrader and Sturtevant have attempted a reconciliation of the cases of *Drosophila* and the bee by use of Goldschmidt's algebraic formulation. They assign a positive value, e.g., +2, to each A and a negative value e.g., -6, to each X. It is then assumed that the effective relation is the algebraic sum of the values of X and A, as given in the column to the right in Table I. On this view the haploid might be a male. But this system has a difficulty in that the intervals between successive indices do not correspond very well with the observed differences between the sex grades. Thus the smallest observed interval in fact, that between the 3N and 2N individuals, is represented by a difference of 4 units, while the very great interval between the male and the female is represented by only six units. At that time the 4N type was not known; and when it is added to the series, the fit is very poor on the algebraic system and very good on the ratio system. I repeat that I do not regard the case of the bee as interpretable on the same basis as *Drosophila* so long as the present account of the mechanism for the bee is unchallenged.

At present the difference between haploid and diploid sexes must be referred to the same type of determination as that responsible for the larger size, rougher texture of eyes and other slight changes that distinguish the 3N from the 2N individual.

But outside of the cases like that of the bee, it seems probable that the ratio type of interaction is the general mode. In evidence of this may be cited the vast array of monoecious plants in which the sex relations in the triploid and tetraploid remain the same as in the diploid. Of course there is high sterility in the triploid forms on account of the instability of the 3N group in meiosis, and the consequent production of inviable gametes or zygotes. Among the most striking confirmations of this ratio view of genic balance is seen in the mosses, through the brilliant work of the Marchals, Schweitzer and von Wettstein. For example, they find in a moss with separate sexes that a 2N gametophyte that combines 2 female groups of chromosomes is a pure female, like the haploid female plant (Table II). Likewise the 2N gametophyte that combines two male groups of chromosomes is a pure male plant, like the haploid male plant. But a 2N gametophyte that combines a male and a female group is no longer

TABLE II
Sex Types in a Dioecious Moss

Sex Type	X (100)	X' (50)	A (80)	Ratio (X + X'):A	Sex Index
Female { 2N	2	—	2	200:160	.125
N	1	—	1	100:80	.125
Proterogynous Herm. 3N	2	1	3	250:240	1.04
Protandrous Herm. { 4N	2	2	4	300:320	.94
2N	1	1	2	150:160	.94
* Protandrous Herm. 3N	1	2	3	200:240	.83
Male { 2N	—	2	2	100:160	.63
N	—	1	1	50:80	.63

* This type is not reported as realized.

a single-sexed plant but is a protandrous hermaphrodite. Furthermore, the 4N gametophyte that combines 2 male chromosome groups with 2 female groups (FFMM) is a hermaphrodite like the FM hermaphrodite. But a triploid form in which two groups are female and one is male is a hermaphrodite that is strongly protogynic instead of protandrous. On the other hand, in working with monoecious mosses where the haploid group is a hermaphrodite, then all haploid, diploid, triploid and tetraploid plants were hermaphrodite without distinction, as they should be from their possession of the same ratio of female to male determiners.

A series of sex-indices, similar to those for *Drosophila*, can be fitted to the dioecious mosses, as shown in Table II. Here it is assumed that there is a pair of chromosomes, X and X', whose difference accounts for the difference between the female and male types. It is assumed that in both these sexes the net effect of the other chromosomes, that may be represented by A, is male-determining. Then, since the X,A type is a female, the value for A must be less than that of X, e.g., $X=100$ and $A=80$. Likewise, since X',A is a male, the value X' must be less than that of A, e.g., $X'=50$. Also, since the FM plant is a hermaphrodite that resembles the normal male more than the normal female, $X+X' < 2A$. And, since the FFM plant is a hermaphrodite that resembles the normal female more closely, $X+X'+X' > 3A$. We have thus five limiting equations for the three values, X, X', and A. As the table shows, the assigned values of 100, 50 and 80 are possible,

although there may be other slightly different values that would give a set of indices whose intervals would correspond even more closely to the observed differences than to those given.

The same conformity to the ratio rule seems to be true in the haploid, diploid, triploid and tetraploid daturas, as far as I can gather. But on the genic balance view each of the twelve kinds of chromosomes of *Datura* might have a distinctive internal unbalance of the sex-controlling genes, similar to the unbalance in the fourth chromosome of *Drosophila*. In *Datura* there is a full series of forms that differ from the 2N by the addition of a particular extra chromosome. If any of these twelve kinds of chromosomes contain more effective male tendency genes than female tendency genes, or vice versa, then one may well expect to discover that some of Blakeslee's "Apostles" and "Acolytes" have atypical sex-relations.

LITERATURE

- Blakeslee, A. F. 1922 Variations in *Datura*, due to changes in chromosome number. *Am. Nat.*, vol. 56, pp. 16-31.
- Bridges, C. B. 1922 The origin of variations in sexual and sex-limited characters. *Am. Nat.*, vol. 56, pp. 51-63.
- Goldschmidt, R. 1920 Untersuchungen über Intersexualität. *Zeit. f. ind. Abst. u. Verer.*, vol. 23, pp. 1-197.
- Morgan, L. V. 1922 Non-criss-cross inheritance in *Drosophila melanogaster*. *Biol. Bull.*, vol. 42, pp. 267-274.
- Schweitzer, J. 1923 Polyploidie und Geschlechterverteilung bei *Splachnum sphaericum* Schwartz. *Flora*, vol. 116, pp. 1-72.
- Wettstein, F. v. 1924 Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage. *Zeit. f. ind. Abst. u. Verer.*, vol. 33, pp. 1-236.



The Effects of Unequal Crossing Over at the Bar Locus in *Drosophila*

A. H. STURTEVANT

Reprinted by author's and publisher's permission from *Genetics*, vol. 10, 1925, pp. 117-147.

Very often in biological research the greatest contribution to our knowledge of a process comes from the discovery of events resulting in its disruption. Thus, the discovery of mutations gave us considerable knowledge concerning normal gene action, and the discovery of lethal mutations provided information about the process of mutation itself.

In Sturtevant's paper we see this kind of event. Although the author set out to study mutation, he encountered a serious disruption of an ordinary process, and thereby made a discovery that has led to considerable alteration in the theory of the gene. In fact, some authors have gone so far as to suggest that the classical idea of the gene needed to be jettisoned completely as a consequence of the events described in this paper. The disrupted process was that of "crossing-over," which is the mechanism whereby equal parts of homologous chromosomes are exchanged during meiosis. Sturtevant found that the parts exchanged were not always equal, and that the consequences of the inequality of exchange were striking indeed.

In Sturtevant's summary you will find the statement that ". . . two genes lying in the same chromosome are more effective on development than the same two genes when they lie in different chromosomes." This concept, and extensions of it, is now known as "position effect" (a term used by Sturtevant in this paper, page 141), and it has destroyed the older concept of genes as "beads on a string," which implies that where the gene is makes no difference in the way it works. Sturtevant's work puts a new emphasis on the role of the chromosome in nuclear control of cellular metabolism, a role perhaps as important as that of supervising the permanence of equality in distribution of genes at the time of meiosis.

INTRODUCTION

IF ONE THINKS OF MUTATIONS AS being simply inherited changes, it be-

comes necessary to distinguish changes that involve whole chromosomes (e.g., non-disjunction or tetraploidy), changes that involve several adjacent

genes (deficiencies and duplications), and what have been called "point-mutations" or "gene-mutations." Probably this last type includes quite diverse processes. It is therefore important to collect information as to the nature of specific examples of mutation. For this purpose it will commonly be necessary to work with a frequently recurring mutation. Only one frequently mutating gene has hitherto been discovered in *Drosophila*, namely, bar. Crossing over has proved to be the key to the mutation behavior of bar, as will be shown in the present paper. The case appears not to be, strictly speaking, a point-mutation after all, but a new kind of section mutation, in which the section concerned is so short as to include only a single known gene, and in which unequal crossing over furnishes the mechanism for bringing about the new types.

HISTORICAL

In 1914 Tice (1914) found a single male of *Drosophila melanogaster* that had narrow eyes (see figures 1 and 2). The new type, called bar (or "barred" in the earlier literature), was found to depend on a sex-linked gene located at 57.0 in the X chromosome. It was further found that the bar character is dominant, in the sense that females carrying one bar gene have eyes distinctly different from the wild-type or "round" eye (figure 3). Because of this dominance the type has been extensively used in linkage experiments. May (1917) reported that the bar gene occasionally reverts to normal (figures 4 and 5)—a process that has more recently been extensively studied by Zeleny (1919, 1920, 1921). Zeleny found that the frequency of reversion is variable, but in many stocks is such that about 1 in 1600 offspring from a pure bar stock receives a not-bar, or

round, allelomorph. Zeleny also concluded that the reversion probably occurs chiefly (or perhaps exclusively) in females. His argument, based on the sex ratio found among reverted individuals, is not as convincing as the direct tests that will be described in this paper, and which verify his conclusion. Zeleny also found that homozygous bar gives rise to a new and more extreme allelomorph of bar, that he has called "ultra-bar." For reasons that will be developed in this paper, I prefer to call it "double-bar." The eyes of double-bar are distinctly smaller than those of bar (figure 6). Zeleny has shown that the type is more strongly dominant over round than is bar, and also that double-bar is largely dominant over bar.

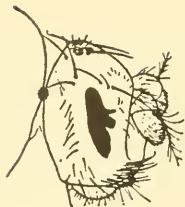
Zeleny likewise found that homozygous double-bar stocks revert to round with a frequency not very different from that of homozygous bar stocks, and that double-bar occasionally mutates to bar; that is, it can go all the way back to round at one step, or it can give bar, which, in turn, is capable of reverting to round. Zeleny has argued that the three types, round, bar, and double-bar, have the same characteristic properties, regardless of their origin. The round eye of reverted bar is indistinguishable from wild-type; bar derived from double-bar does not differ from the original bar, etc. This point will be considered in more detail in a later section.

Sturtevant and Morgan (1923) showed that double-bar over bar¹ also gives rise to round-eyed individuals. They reported three reversions from this combination and three from ho-

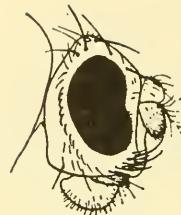
¹ In this paper the constitution of heterozygotes will be expressed as above in order to avoid circumlocution or indefiniteness. "Double-bar over bar" is to be understood as: "carrying double-bar in one X chromosome and bar in the other X chromosome."



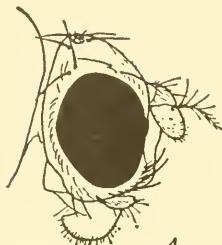
1



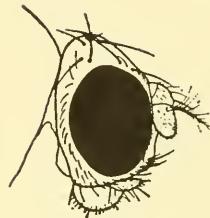
2



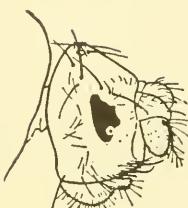
3



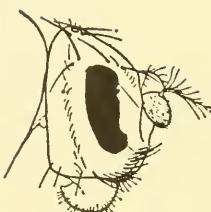
4



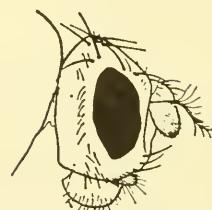
5



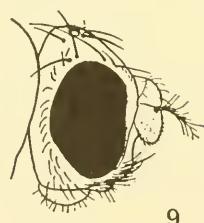
6



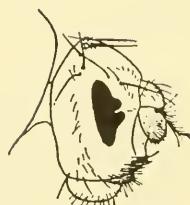
7



8



9



10

Figs. 1–10. (1) Homozygous bar female. (2) Bar male. (3) Bar-over-round female. (4) Female homozygous for round, that was obtained by reversion. (5) Male that carries round, obtained by reversion. (6) Double-bar male. (7) Homozygous infrabar female. (8) Infrabar male. (9) Infrabar-over-round female. (10) Double-infrabar male.

mozygous bar. In all cases the mothers had been heterozygous for forked,² which lies 0.2 units to the left of bar, and for fused³ which lies 2.5 units to the right of bar. All six reversions represented crossovers between forked and fused, though the total forked fused crossovers constituted less than 3 percent of the number of individuals examined. Sturtevant and Morgan also reported that experiments in which bar entered only through the males had failed to give any reversions, though no numerical data were reported. The present paper is based on the results of a more detailed study of the relations first shown by Sturtevant and Morgan (1923).

MUTATIONS AND CROSSING OVER

The results from homozygous bar females, that were reported by Sturtevant and Morgan, were from females of the constitution $\frac{+ B f_u}{f B +}$. A more efficient type of experiment is that in which females of the constitution $\frac{+ B +}{f B f_u}$ are mated to forked bar fused males. Table 1 (first row) shows the results obtained from an extensive series of this type. In the second row of table 1 are given the results from mating a few females of the above constitution to forked fused males.

TABLE 1

$$\frac{B}{f B f_u} \text{♀} \times f B f_u \text{♂ (1st row)} \text{ or } f f_u \text{♂ (2nd row)}$$

Type of Male Used	Bar								Total	Round				Double- Bar	
	♀				♂					♀	♂	♀	♂	♀	♂
	0*	1		0		1		f _u	f	+	f _u	f	f	f	
fBf _u	5413	3749	94	140	5218	4160	93	124	18991	0	2	1	2	1	1
ff _u	374	359	6	6	352	324	10	7	1438	1	0	0	1	0	0
Total	5787	4108	100	146	5570	4484	103	131	20429	1	2	1	3	1	1

* In this and the following tables "0" signifies non-crossover classes; "1," classes resulting from crossing over in the first region, etc.

The mutant females that appeared in these experiments were, of course, all heterozygous for whichever allele-morph (bar or round) they received from their fathers, but in tables 1 to

18 this paternal gene is ignored and the females classified according to the maternal gene. In all doubtful cases (including both double-bars), the classification was checked by raising offspring from the mutants, since there is sometimes difficulty in classifying single individuals, a difficulty that can be removed by examination of a series of specimens of a given constitution.

It will be observed that seven of the

² Forked is a recessive bristle modification. Locus 56.8 in the X chromosome (see Morgan and Bridges 1916).

³ Fused is a recessive venation character. Its locus is at 59.5 in the X chromosome (see Morgan and Bridges 1916, Lynch 1919).

eight reversions, and also both of the double-bars, occurred in gametes that came from crossing over between forked and fused, though the total forked fused crossovers constituted only 2.4 percent of the population. The one exceptional case, a wild-type male, is not above suspicion of having arisen through contamination rather than reversion of bar. That he was really round-eyed was proven by tests. Only 4 other exceptions to the rule that mutation in this locus is accompanied by forked-fused crossing over have been met with in the work here reported. These will be discussed separately later.

On the basis of these results we may formulate the working hypothesis that both reversion and the production of double-bar are due to *unequal crossing over*. If we suppose that, in a female $+ B +$, $f B f_u$, crossing over occurs in such a way that the respective points of interchange lie to the left of the bar locus in one chromosome, but to the right of it in the other one, there will result chromosomes of the constitution $fBB+$ and $+f_u$ (or $f+$ and $+BBf_u$). The hypothesis is that reverted round is simply *no-bar*, and that double-bar is *BB*,—this being the reason for abandoning Zeleny's name, ultra-bar.

This hypothesis makes reverted round and double-bar complementary crossovers, and they should accordingly be produced with equal frequency. Table 1 agrees with Zeleny's more extensive data in showing that round is apparently far more frequent than double-bar; but such a result was to be expected for two reasons. Double-bar is not as viable as round, so that fewer of the double-bar mutant individuals would be expected to survive; and double-bar is not always clearly distinguishable from bar, so that some

mutant individuals are probably overlooked, while it is not likely that any reversion is overlooked through difficulty of classification.

The double-bar over bar experiments reported by Sturtevant and Morgan (1923) can be interpreted in the same way: the reversion is here due to unequal crossing over just as in homozygous bar. In the earlier cultures of the experiments previously reported, only the reversions were classified for forked and for fused. Two of the reversions were in such incompletely classified cultures. Table 2, including all the double-bar over bar data for which complete counts are available, contains one of the previously reported reversions and one new one. This table includes only the male offspring, since the *BB* derived from the fathers rendered the classification of the females uncertain.

The process of unequal crossing over might be expected to give rise to triple-bar from the females that are double-bar over bar. No individual that could be so identified was obtained, though several specimens with very small eyes were tested. All those that were fertile proved to be double-bar. Apparently triple-bar is either inviable or sterile. This problem will be discussed again below.

TABLE 2

$$\frac{B}{fBBf_u} \text{♀} \times fBBf_u \text{♂}$$

B or BB ♂				Total	Round ♂
0		1			f
+	ff_u	f_u	f		
3933	2741	62	73	6809	2

My own experiments with homozygous double-bar have not yielded any mutations, probably because I

have found this type hard to breed and have therefore not obtained large numbers of offspring. It may be recalled that Zeleny has obtained both bar and round from such females, but not in experiments in which forked and fused were present. In table 3, showing the data I have obtained, only males are recorded, for the same reason as in table 2, and also because the females could not be classified for fused.

TABLE 3

$$\frac{fBB}{BBf_u} \text{♀} \times fBB \delta$$

BB δ		Total
0	1	
f _u	f	
627	956	1644
37	24	

In the case of double-bar over round, bar should be produced by any crossover between the two bars of the double-bar chromosome. This event might seem less unlikely to occur than the type of unequal crossing over invoked in the preceding experiments; and both of the chromosomes resulting from such crossing over should yield bar, whereas in the preceding cases, a given crossing over must always have yielded chromosomes bearing two different kinds of bar allelomorphs. It is accordingly in agreement with the hypothesis that tables 4 and 5 show a higher percentage of mutation than do tables 1 and 2. Two non-disjunctive individuals ($a ff_u \delta$ and a $+++ f BB f_u \text{♀}$) have been omitted from table 5.

No mutations are to be expected from females that are heterozygous

TABLE 4

$$\frac{BB}{ff_u} \text{♀} \times ff_u \delta$$

Sex	Double-Bar and Round						Total	Bar		
	0		1		2			f _u	f	
	BB	ff _u	f _u	fBB	BBf _u	f				
♀	1174	784	1	1	18	30	2018	0	1	
♂	993	943	1	1	14	19	1971	1	1	

for bar and for round $\left(\begin{matrix} B \\ + \end{matrix}\right)$, since crossing over can not produce any new combination. The results of the tests are shown in tables 6 and 7, and are in agreement with this expectation. One non-disjunctive female $\left(\begin{matrix} + + + \\ fBf_u \end{matrix}\right)$ was also produced in this series.⁴

L. V. Morgan (1922) has described a race of *D. melanogaster* in which the two X chromosomes of the female are attached to each other, so that such a

would be either a double crossover—which is not at all probable in such a short chromosome section, or a non-crossover reversion of bar. This female was mated to an unrelated bar male, and produced 124 bar-over-round daughters and 96 round-eyed sons; but none of the sons showed either forked or fused. This result must mean that the exceptional female was due to contamination.

⁴ This series also produced one female that was wild-type in appearance. Such a female

TABLE 5

$$\frac{+}{fBBf_u} \text{♀} \times ff_u \text{♂}$$

Sex	Double-Bar and Round						Total	Bar		
	0		1		2			f _u	f	
	+	fBBf _u	BBf _u	f	f _u	fBB				
♀	464	392	0	1	14	23	894	1	0	
♂	495	406	0	0	6	10	917	1	1	

female gives 100 percent non-disjunction. This race, and others separate in origin but having the same peculiarity of attached X's, have been used to test the mutability of bar in the male. If a round-eyed female with attached X's is crossed to a bar male, all the sons get their X chromosomes from the bar father and accordingly furnish a direct

test of the mutability of bar in males.⁵ A total of 10,079 bar males has been observed from such matings, with no rounds or double-bars. There was, however, one other male that had an eye intermediate between bar and round. This new type, called infrabar (figures 7, 8, 9) has been shown to represent a new allelomorph of bar. Its

TABLE 6

$$\frac{+}{fBf_u} \text{♀} \times ff_u \text{♂}$$

Sex	Bar						Total	
	0		1		2			
	+	fBf _u	Bf _u	f	f _u	fB		
♀	331	276	0	0	12	13	632	
♂	316	265	0	1	10	11	603	

somatic appearance will be described in more detail later in this paper.

Tests soon showed that infrabar behaved as a single unit in inheritance. Bar can not be recovered from it, and it shows the same linkage relations as bar. The convincing proof that it represents a modification of the bar gene will appear below.

Homozygous infrabar behaves like bar in that it reverts to normal, and also produces a new and more extreme type, double-infrabar (figure 10), analogous to double-bar. As shown in

table 8, both these events are again associated with forked-fused crossing over.

Of the three double-infrabar individuals, one was sterile, and one was accidentally lost, but the constitution of the other was established by breed-

⁵ Occasionally the attached X's separate, and a regular son carrying a maternal X is produced. In the present series of experiments this source of error was eliminated by having the two maternal X's differ from the paternal one in at least one other mutant gene besides bar.

TABLE 7

$$\frac{B}{ff_u} \text{♀} \times ff_u \text{♂}$$

Sex	Bar						Total	
	0		1		2			
	B	ff _u	f _u	fB	Bf _u	f		
♀	1792	1356	3	7	36	41	3235	
♂	1523	1334	4	3	26	36	2926	

ing tests. The type was also obtained in another experiment; and its appearance and mutation behavior will be described later. This series shows the same excess of rounds over double

forms as did the bar series, and the explanation is doubtless the same. The difficulty of separation is even greater here, owing to a greater variability in eye size (see below).

TABLE 8

$$\frac{Bi}{fBi f_u} \text{♀} \times f Bi f_u \text{♂}$$

Sex	Infrabar				Total	Round		BiBi		
	0		1			f _u	f	f _u	f	
	+	ff _u	f _u	f						
♀	4607	3606	94	142	8449	0	5	0	1	
♂	4341	3905	97	106	8449	2	11	2	0	

Bar-over-infrabar females of three different constitutions have been tested. They have produced rounds, and also a new double type that had eyes only very slightly larger than

those of double-bar. These must evidently have bar and infrabar in the same chromosome. Tables 9, 10 and 11 show these results. In most cases the fathers of these cultures did not carry

TABLE 9

$$\frac{f Bi}{B f_u} \text{♀} \times \text{various } \delta \text{♂}$$

Bar and Infrabar Males				Total	Double Type			
0		1			f _u	δ		
f _u	f	+	ff _u			+	ff _u	
623	682	26	18	1349	?	1	1	

fused; for this reason the females, among which no mutants were de-

tected, are not listed. The forked fused mutant male was sterile, but the con-

stitution of the other was tested. All the experiments here described, on "bar-infrabar" (figure 10), concern this mutant gene. The stability of this new type in the male has also been

tested. Matings of bar-infrabar males to attached-X females (also differing in at least one other sex-linked gene) have produced 9042 non-disjunctional sons,—all of them bar-infrabar.

TABLE 10

$$\frac{B^i}{f B f_u} \text{♀} \times f B f_u \text{♂}$$

Sex	Bar and Infrabar				Total	Mutants		
	0		1					
	+	ff_u	f_u	f				
♀	497	415	10	18	940	0		
♂	505	476	15	16	1012	0		

The double-type male of table 11, which resembled those from table 9, was tested. His descendants are dis-

cussed below under the name "infrabar-bar."

These new double types have made

TABLE 11

$$\frac{B}{f B^i f_u} \text{♀} \times f B^i f_u \text{♂}$$

Sex	Bar and Infrabar				Total	Round		Double Type	
	0		1			f_u	f		
	+	ff_u	f_u	f					
♀	2445	2203	59	74	4781	0	1	0	
♂	2365	2347	64	71	4847	1	0	1	

it possible to devise crucial tests of the theory of unequal crossing over, which may now be described. The

first test made was that of bar-infrabar over round. Tables 12 and 13 show the results obtained. In addition one

TABLE 12

$$\frac{f B B^i}{f_u} \text{♀} \times f f_u \text{♂}$$

Sex	Bar-Infrabar and Round						Total	Bar	Infrabar	
	0		1		2					
	f_u	$f B B^i$	$B B^i$	ff_u	+	$f B B^i f_u$		ff_u	+	
♀	303	394	4	0	14	8	723	0	1	
♂	321	308	0	2	7	3	641	1	1	

culture of this series gave two wild-type females, one in the first count and another in the last count, ten days later. These females give rise to the same difficulties as did the wild-type obtained in the series reported in table 7. One of them was tested, and gave unexpected results. The mother had

TABLE 13

$$\frac{BB^i}{f f_u} \text{♀} \times ff_u \text{♂}$$

Sex	Bar-Infrabar and Round						Total	Bar	Infrabar	
	0		1		2			f _u	f	
	BB ⁱ	ff _u	f _u	fBB ⁱ	BB ⁱ f _u	f				
♀	2549	1684	3	1	43	74	4354	0	0	
♂	2322	1857	5	8	48	63	4303	2	4	

tional individuals were not due to contamination. They will be discussed again later.

Tables 12 and 13 show that bar and infrabar can both be recovered from bar-infrabar. It appears then that in the double form the individual elements maintain their identity. Even more important, however, is the indication that they maintain their sequence in the chromosome. As shown in table 9, the bar-infrabar first came from the combination $\frac{f B^i}{B f_u}$, as a not-forked not-fused male. If the two elements of double forms are arranged in

the same linear series as the rest of the genes, this result must mean that the bar now lies to the left of the infrabar. This supposition is entirely borne out by tables 12 and 13, which are experiments of the usual type used to establish sequence of genes. All the 9 single types recovered agree with the supposed sequence.

Bar-infrabar has also been tested against infrabar (table 14).

Here again there is an opportunity for the production of a triple form,—but since the corresponding round did not appear, its absence is not significant. The one double-infrabar con-

TABLE 14

$$\frac{BB^i}{f B^i f_u} \text{♀} \times f B^i f_u \text{♂}$$

Sex	BB ⁱ and B ⁱ						B ⁱ B ⁱ	
	0		1		2			
	BB ⁱ	fB ⁱ f _u	B ⁱ f _u	fBB ⁱ	BB ⁱ f _u	fB ⁱ		
♀	850	702	3	3	17	28	1603	0
♂	867	860	4	2	22	23	1778	1

firms the sequence of the component parts of bar-infrabar. It was tested, and all the results from double-infrabar reported below were obtained from flies descended from it.

Examination of the data in table 11

shows that the double form obtained there must have had infrabar to the left of bar; that is, it was infrabar-bar instead of bar-infrabar. The tests made with it appear in table 15.

These results show, in fact, that the

TABLE 15

$$\frac{fB^iB}{f_u} \text{♀} \times ff_u \text{♂}$$

Sex	Infrabar-Bar and Round						Bar	Infrabar		
	0		1		2					
	f_u	fB^iB	B^iB	ff_u	+	fB^iBf_u				
♀	483	469	1	1	15	15	984	2		
♂	478	408	1	9	17	15	928	0		

sequence is infrabar-bar as supposed. Except for this difference the mating is the same as in table 12. It will be observed that the two not-forked not-fused single types in that table were both infrabar, while the two obtained here were bar; the one forked fused there was bar, here it was infrabar. There is a total of 13 single-type

mutants in tables 12 to 15, all of them agreeing in indicating that the two elements of double types maintain not only their individuality but their sequence.

The double-infrabar obtained in table 14 has been tested against round (tables 16 and 17).

The results in these tables show that

TABLE 16

$$\frac{fB^iB^i}{f_u} \text{♀} \times ff_u \text{♂}$$

Sex	B ⁱ B ⁱ and Round						Total	B ⁱ		
	0		1		2					
	f_u	fB^iB^i	B^iB^i	ff_u	+	$fB^iB^if_u$				
♀	538	629	1	1	12	17	1198	0		
♂	574	505	1	1	13	7	1101	2		

double-infrabar was correctly identified, and that it behaves as was to be expected, giving infrabar by both kinds of crossing over,—just as double-bar gives bar in both cases.

Double-infrabar over infrabar has also been tested, in the hope of obtain-

ing triple-infrabar (table 18).

No triple-infrabar was detected; but its absence is not surprising, since the corresponding round occurred only once, and since it is not at all sure that the triple form could be distinguished from the double one.

TABLE 17

$$\frac{B^i B^i}{f f_u} \text{♀} \times f f_u \text{♂}$$

Sex	Double Infrabar and Round						Infrabar	
	0		1		2		Total	f_u
	$B^i B^i$	$f f_u$	f_u	$f B^i B^i$	$B^i B^i f_u$	f		
♀	1664	1404	3	3	39	37	3150	1
♂	1449	1193	3	3	29	42	2719	1
								2

One further type of female was tested, namely, bar-infrabar over infrabar-bar (table 19).

The one mutant infrabar obtained in the series is in agreement with the sequence in which bar and in-

TABLE 18

$$\frac{B^i B^i}{f B^i f_u} \text{♀} \times f B^i f_u \text{♂}$$

Sex	Double Infrabar and Infrabar *					Round	
	0		1		Total	f	
	+	$f f_u$	f_u	f			
♀	851	777	25	31	1684	1	
♂	665	750	26	29	1470	0	

* The double-infrabar and infrabar flies are not entered separately. In the original counts they were separated; but the classification is uncertain at times.

frabar were supposed to lie in the two X chromosomes of the mother; and this mutant is also the only one yet

obtained from a mother carrying a double-type allelomorph in each X, where forked and fused were hetero-

TABLE 19

$$\frac{BB^i}{f B^i B f_u} \text{♀} \times f B^i B f_u \text{♂} \text{ (1st row), or } f f_u \text{♂} \text{ (2nd row)}$$

	BB ⁱ and B ⁱ B								B ⁱ	
	♀				♂					
	0		1		0		1			
	+	$f f_u$	f_u	f	+	$f f_u$	f_u	f	♂	
1	95	74	3	4		
2	428	335	8	15	341	319	7	16	176 1469	
									0 1	

* Females not counted in the mating to $B^i B$ male.

zygous. It therefore serves to complete the demonstration of the relation of crossing over (between forked and fused) to mutation in the bar locus.

The experiment of table 19 was, however, planned for another purpose. It will be seen that in the mother, which was $\frac{B\ B^i}{B^i\ B}$, equal crossing over might give rise to new types, namely, double-bar and double-infrabar. The first could not be distinguished, in somatic appearance, from the unmutated double types (BB^i and B^iB); but the double-infrabar should be readily detected. Such an individual would be forked. It may accordingly be concluded that none of the 35 forked (not-fused) offspring represented equal crossing over between the halves of the two double-type bar allelomorphs present. It therefore seems probable that crossing over of this kind is not much, if any, more frequent than is that between the two elements of a double-type allelomorph when the other chromosome carries round (tables 4, 5, 12, 13, 15, 16).

Several of the above tables agree with a small series of infrabar over round, heterozygous for forked and for fused, in showing that infrabar lies between forked and fused. It must clearly be either an allelomorph of bar, or bar plus a modifier that lies near bar.

The experiments with bar-infrabar and with infrabar-bar show that these two types both contain infrabar as a unit distinct from bar.

Since bar-infrabar was produced by an unequal crossover that occurred very close to the left of infrabar, it becomes unlikely that a modifier can lie on that side of the bar locus; infrabar-bar furnishes similar evidence that there is no modifier to the right. All the evidence thus indicates clearly that the infrabar gene is really a modification of the bar gene itself.

FREQUENCY OF BAR MUTATIONS

The data presented in tables 1 to 19 have been examined in an attempt to formulate some general statements as to the relative frequency of the various types of mutation in the bar locus. It is probable that homozygous double types, and double over single show the lowest frequencies of mutation, and that double type over round shows the highest. Both these results might have been expected. There is, however, so much variability among crosses of the same general nature that these conclusions must be accepted with caution. For example, the two largest series are those from homozygous bar (20,438 offspring) and from homozygous infrabar (16,918 offspring). The mechanical conditions should be alike in the two cases, since both represent homozygous single types. Yet from the first there appeared 0.03 percent of reversions, or 1 in each 2920 offspring; from the second there were 0.11 percent, or 1 in 940 offspring. In view of such unexplained differences as this, and in view of the statistical difficulty of determining probable errors for such small percentages, it does not seem profitable to discuss further this aspect of the data, except to note that mutation frequency does not appear to be correlated with frequency of forked-fused crossing over.

THE CROSSOVER VALUES FOR FORKED, BAR AND FUSED

The experiments recorded in tables 1 to 19 include by far the largest series of data yet accumulated for the crossover values of the three loci, forked, bar and fused. These are summarized in tables 20 and 21. In these tables all the mutant individuals have been omitted. Their inclusion would not have affected any of the values appreciably. In table 22 the data already

TABLE 20
fBf_u crossing over

Type of Female Tested	Non-Crossovers	Crossovers		Total
		Region 1	Region 2	
Single type over round ..	7,193	18	185	7,396
Double type over round ..	22,937	52	603	23,592
Double type over single ..	3,279	12	90	3,381
Total	33,409	82	878	34,369
Percentage	97.21	0.24	2.55	

published on these loci are included with the above, in order to arrive at final "map values" for the three loci.

On the basis of these data, it seems best to map forked 0.2 unit to the left of bar, that is, at 56.8; and fused 2.5 units to the right of bar, at 59.5, thus making the forked-fused interval 2.7 units.

FACET NUMBER

It has been shown by Zeleny and Mattoon (1915), May (1917) and Zeleny (1922) that selection for number of facets is effective in isolating lines of bar flies with high facet numbers or with low. Though no detailed genetic analysis has yet been reported, there is abundant evidence in these

TABLE 21
ff_u crossing over

Type of Female Tested	Non-Crossovers	Crossovers	Total
Single type over single	48,966	1290	50,256
Double type over single	9,717	246	9,963
Double type over double	3,175	114	3,289
Total	61,858	1650	63,508
Percentage	97.40	2.60	

papers that ordinary bar stocks are heterogeneous for modifiers (not in the bar locus) that affect facet number. This is also the impression I have gained from extensive but less exact studies, with numerous crosses involving bar.

Another source of variability in facet number is temperature. It was shown by Seyster (1919) that high temperature decreases the facet number of bar, and this relation has been studied in great detail by Kafka

(1920), Zeleny (1923), A. H. Hersh (1924) and R. K. Hersh (1924). These observers have shown that the effect is present, though in varying degree, in double-bar, round, and in various heterozygotes, as well as in bar. Although these studies furnish essential data for any complete analysis of the mode of action of the bar series of allelomorphs upon development, they need not be further discussed here.

The evidence just reviewed indicated that it would be necessary to get

TABLE 22
Total linkage data

Loci	Source of Data	Crossovers	Total	Per Cent
<i>fB</i>	Morgan and Bridges 1916	8	1,706	0.5
	Bridges 1917	5	980	0.5
	Table 20	82	34,369	0.2
Total		95	37,055	0.26
<i>ff_u</i>	Weinstein 1918	200	8,298	2.4
	Table 21	1650	63,508	2.6
	Total "primary" data		71,806	2.58
	Morgan and Bridges 1916	37	1,201	3.1
	Table 20	960	34,369	2.8
Grand total		2847	107,376	2.65
<i>Bf_u</i>	Morgan and Bridges 1916	222	8,768	2.5
	Bridges 1917	46	1,401	3.3
	Table 20	878	34,369	2.5
	Total	1146	44,538	2.57

stocks as nearly uniform for modifiers as possible, and also to control the temperature, if any reliable data were to be collected as to the relative effectiveness on facet-number of the various combinations of bar and infrabar. Accordingly, a female that was *fBf_u*,⁶ was mated to a round-eyed vermilion male from vermilion stock. The descendants from this mating were inbred (brother-sister pair matings) for seven generations. In each generation a female heterozygous for two of the three bar allelomorphs concerned was mated to a male carrying the third allelomorph. The line was made homozygous for forked; but both vermilion and fused were eliminated. No other selection was prac-

ticed. A female of the third inbred generation was mated to an infrabar male, and a daughter that was infrabar over round was mated to a male from the fourth generation. For three more generations the infrabar series was crossed to the inbred line. After the eighth generation the pedigrees are somewhat more complex, but as close inbreeding as was compatible with maintaining four allelomorphic sex-linked genes was continued for six more generations before the facet counts were begun. The other types studied (bar-infrabar, reverted bar, etc.) were all crossed to the inbred stock just described, at least five times (mostly using females to allow crossing over and get as much of the X's uniform as possible) before being used in the counts. This procedure should have resulted in making the various stocks practically alike with respect to modifying genes, and the results obtained are sufficiently consistent to indicate that there was no heterogeneity

⁶ Derived from the experiments of table 1, so that the *BB* of the following discussion is known to have come from the *BB* male of that table, and to have been derived from homozygous bar by forked-fused crossing over.

in major modifiers, though it is still possible to interpret some of the minor differences observed as being due to uneliminated diversity in modifiers.

The temperature control used was not very exact, but maximum-minimum daily records show that 25°C was maintained to within about $\pm 1^\circ\text{C}$, and even these deviations were probably of short duration. For the main body of the experiments it has not proved possible to detect any systematic effect of the fluctuations in temperature that did occur. The few experiments in which such an effect is perhaps present will be specified when described.

The facets of the smaller eyes,—up to and including homozygous bar,—were counted directly under the binocular microscope, usually on etherized flies, but in some cases on alcoholic specimens. The eyes larger than this were not found to be workable by this method. Such specimens were killed and cleared in KOH. The surface of each eye was then removed and mounted on a slide. By the aid of a camera lucida a drawing was made, representing each facet by a dot, and these dots were then counted, each dot being marked by a check as counted. In all cases the right eye alone was used.

The main series of data is shown in table 23.

The table shows that homozygous infrabar is about like bar over round in facet number, but the two types can be separated by a peculiarity common to all the larger infrabar and double-infrabar types, namely, a roughened appearance of the eye, due to irregularities in the rows of facets. This peculiarity is not present in bar eyes, and is almost completely recessive in bar over infrabar. In infrabar over round (which is not far from round in facet number) the roughness is vari-

able in extent, and may be not at all evident,—in which case the type can not be distinguished with certainty from homozygous round. In other stocks, where the modifiers are different, it often happens that infrabar over round is regularly conspicuously roughened and is easily distinguishable from round. This roughness of the eyes may be taken as evidence that the infrabar gene is qualitatively different from bar, rather than being merely a fraction of bar.

The table shows that in general when bar and infrabar are both present in an individual, the infrabar produces almost as great an effect in reducing facet number as would another bar, though in the absence of bar the infrabar is far less effective. For example, $\frac{B}{B} = 68$, $\frac{B^i}{B^i} = 348$; but $\frac{B}{B^i} = 74$. And in general, BB^i is practically as effective as BB throughout the table. In two cases the observed differences, though surely not significant, indicate that BB^i is more effective than BB (that is, the combinations with BB and with B^i). Similar relations are shown in other parts of the table.

The most striking relation shown by table 23 is that the relative position of identical genes affects their action on facet number. There are three similar comparisons to be made:

$$\frac{B}{B} = 68.1 \quad \text{versus} \quad \frac{BB}{+} = 45.4$$

$$\frac{B}{B^i} = 73.5 \quad \text{versus} \quad \frac{BB^i}{+} = 50.5$$

$$\frac{B^i}{B^i} = 292.6^7 \quad \text{versus} \quad \frac{B^iB^i}{+} = 200.2$$

⁷ This value for $\frac{B^i}{B^i}$ is different from the one of table 23. It is based on a series reared at the same time as the $\frac{BB^i}{+}$ with which it is here compared. The difference between this value and that of the table is probably due to temperature.

TABLE 23

Facet numbers of flies carrying various combinations of bar allelomorphs

		BB	BB ⁱ	B ⁱ B ⁱ	B	B ⁱ	+
BB	n	47					
	M	24.96±.30					
	σ	3.03±.21					
BB ⁱ	n	20	51				
	M	24.10±.35	26.69±.29				
	σ	2.36±.25	3.07±.21				
B ⁱ B ⁱ	n	25	32	27			
	M	26.68±.29	27.91±.36	38.19±.71			
	σ	2.13±.20	2.98±.25	5.48±.50			
B	n	51	86	28	50		
	M	36.43±.30	37.01±.25	38.29±.59	68.12±1.09		
	σ	3.18±.21	3.49±.18	4.64±.42	11.15± .77		
Bi	n	30	53	3	30	18	
	M	41.77±.41	37.79±.52	138.2	73.53±1.29	348.4±12.4	
	σ	3.34±.29	5.62±.37	—	10.52± .92	78.0± 8.8	
+	n	161	70	27	25	25	25
	M	45.42±.24	50.46±.40	200.2±8.6	358.4±7.9	716.4±3.4	779.4±4.1
	σ	4.57±.17	4.93±.28	66.7±6.1	58.2±5.6	24.9±2.4	30.6±2.9
♂	n	261	247	40	40	25	25
	M	29.02±.17	29.68±.15	45.98±1.19	91.03±1.76	478.1±13.7	738.8±6.5
	σ	4.02±.12	3.94±.10	11.13± .84	16.61±1.25	101.8± 9.7	48.1±4.6

Since the bar allelomorphs are to be thought of as inhibitors of facet development, it will be seen that this comparison indicates that *two bar allelomorphs lying in the same chromosome are more effective than are the same two allelomorphs when they lie in opposite chromosomes.*

Such an unexpected result must of course be checked up carefully. Only two possibilities of avoiding the above conclusion seem open. The results are due to (1) accidental differences in temperature or modifiers; or (2) the round allelomorph of bar brings about a reduction in facet number just as does bar. Both of these possibilities can be eliminated, as the following paragraphs will show.

There is no temperature effect,

since in each case the cultures were reared side by side; and in the case of $\frac{B}{B}$ versus $\frac{BB}{+}$, several different tests all gave the same type of result.

If the results are due to accidental modifiers it is scarcely conceivable that these should lie anywhere but near the bar locus, because of the inbreeding to which the stocks have been subjected. As will be shown below, another bar chromosome (derived by mutation from the inbred BB of this strain) has been found to give results sufficiently close to the bar used here so that the conclusions as to the effect of position must apply also to the new bar. And two other not-bar ("round") chromosomes have been found to give substantially the same result as the one

employed here (see below). These facts eliminate the possibility of explaining the result as due to accidental genetic or environmental differences.

The second possible escape from the conclusion as to the effect of position lies in the assumption of an effect produced by the round allelomorph. This has been tested by determining the effect on facet number of reverted bar and reverted infrabar. Round obtained by reversion from homozygous bar or infrabar stocks cannot carry a normal allelomorph on the view advanced in this paper, unless such an allelomorph is already present in the parent stocks. But such a gene is, almost by definition, not an allelomorph of bar; and in any case cannot be supposed to produce the effects here under discussion,

since the $\frac{B}{B}$ and the $\frac{BB}{+}$ would both carry it.

Two different rounds by reversion have been introduced into the inbred stocks, by the same method as used for BB^i and B^iB^i . Care was taken never to use flies carrying one of these reverted rounds in the same culture with the old round of the inbred stock, so that it is certain that these new rounds are really due to reversion,—if not from the supposed source, then from some of the allelomorphs within the inbred stock, since a new reversion may have occurred during the process of getting the desired modifiers into the reverted round stocks. One of the reverted rounds, called "rev. *B*," came from the homozygous bar experiments of table 1; the other, called "rev. *Bⁱ*," came from the homozygous infrabar experiments of table 8. The results of these tests are shown in table 24.

These data suggest that the rever-
sions,—especially the more thoroughly

TABLE 24

Facet counts to test nature of rever-
sions

	Chromosome Tested Against		
	<i>BB</i>	<i>BBⁱ</i>	<i>Y</i> (i.e., ♂)
Wild-type (control)	45.42 ± .24	50.46 ± .40	738.8 ± 6.5
Rev. <i>B</i>	45.63 ± .45	45.48 ± .47
Rev. <i>Bⁱ</i>	43.92 ± .26	46.13 ± .36	704.4 ± 8.0

tested one from infrabar,—may give slightly smaller eyes than does the wild-type allelomorph. Certainly they do not give larger eyes. And still more certainly, with *BB* or *BBⁱ* they do not give as large eyes as do the correspond-

ing types $\frac{B}{B}$ and $\frac{B}{B^i}$. These data eliminate the second possible method of explaining away the effect of the position of the bar allelomorphs on facet number. The conclusion must stand as stated on p. 137.

It seems probable that such an in-

fluence of the relative position of genes on their effectiveness in development may be interpreted in terms of diffusion and localized regions of activity in the cell. This idea is, however, scarcely worth elaborating until more evidence is obtained. It may, however, be pointed out that there is another possible application of the hypothesis of a position effect.

It has been shown by Bridges (1921) that in triploid individuals the recessive genes brown, plexus and speck do not become dominant to their normal

allelomorphs even when two recessive allelomorphs and one dominant are present. Unpublished data collected by Bridges (in part verified in an independently arisen series of triploids in my own experiments) show that this relation is a general one for allelomorphs that do not produce an obviously intermediate diploid heterozygote. But Bridges (Bridges and Morgan 1923) has shown that a different relation may occur, even for some of the same genes that show the former relation in triploids. In the example referred to, a portion of the second chromosome, carrying the normal allelomorph of plexus (among other genes) has become attached to a chromosome III. It is possible, therefore, to obtain individuals with two complete second chromosomes, each of these carrying the plexus gene, while a normal allelomorph of plexus is present in the section attached to a chromosome III. Such individuals are plexus in appearance,—not as extreme as those in a pure plexus stock, but far more like such a stock than like the ordinary triploids carrying two plexus and one non-plexus allelomorphs. While it is possible that the difference here is due to a different "balance" of modifying genes in the extra section of chromosome II, it seems likely that the effective agent is a difference in position. It is to be remembered that in *Drosophila* the homologous chromosomes lie closely apposed in somatic divisions (see Metz 1916), so that there is probably a real difference in relative positions in the two cases.

Besides the comparison of round (ultimately from vermillion stock) with round obtained by reversion from bar and from infrabar, two other derived types have been compared (as to facet number) with the corresponding original types.

An infrabar from bar-infrabar over round (table 13) was introduced into the inbred strain by six successive backcrosses, and was then compared with the old inbred infrabar, both types being made heterozygous for double-bar. The control (old infrabar) value is rather lower than the value given in table 23, probably because the temperature ran slightly higher.

Since the difference between the two means is about 4.5 times its own probable error, it is probably significant, but more extensive data will be required to establish this point.

A female of the inbred series that was double-bar over round, mated to a round male, gave rise to one bar male by mutation. This male was mated to double-bar-over-round females, and the resulting double-bar-over-bar daughters were compared with double-bar over the old inbred bar, derived from cultures made up at the same time and put side by side. Here again there is a slight difference from the value of table 23, perhaps due to a temperature difference.

The difference between the means is slightly over 3 times its probable error, and may be considered as doubtfully significant. Here again, more data are needed.

It may be pointed out that in tables 25 and 26 the derived type is presumably the larger in both cases. It is possible that this result is to be correlated with that recorded in table 24, namely, that round by reversion is perhaps smaller than wild-type. Both relations are consistent with the view that there exists a normal allelomorph of bar that has an effect on facet number opposite to, but much weaker than, that of bar; for both of the derived single types tested were from double type over round, so that these derived forms

TABLE 25

Facet counts from $\frac{BB}{Bi}$

	Number	Mean	σ	Difference of Means
Old B^i	40	$34.73 \pm .35$	$3.24 \pm .24$	
New B^i	36	$36.79 \pm .31$	$2.76 \pm .22$	$2.05 \pm .46$

TABLE 26

Facet counts from $\frac{BB}{B}$

	Number	Mean	σ	Difference of Means
Old B	31	$33.10 \pm .33$	$2.76 \pm .24$	
New B	30	$34.87 \pm .45$	$3.65 \pm .32$	$1.77 \pm .56$

may really be single type plus normal allelomorph. This possibility requires further experimental investigation.

ARE MUTATIONS IN GENERAL DUE TO UNEQUAL CROSSING OVER?

One of the first problems raised by the discovery of the nature of bar reversion is as to how widespread may be the phenomenon of unequal crossing over. One direct test has been attempted, making use of Muller's method of testing for the frequency of occurrence of new lethal mutations. Females were made up that carried one wild-type X chromosome and one X with the mutant genes scute (locus 0.0), echinus (5.5), crossveinless (13.7), cut (20.0), vermilion (33.0), garnet (44.4), and forked (56.8). Such females were mated to males carrying all the mutant genes named. In such matings it is possible to detect practically all the crossing over that occurs in the X chromosome, except that to the right of forked (about 13 units). Counts were made from individual fe-

males, in order to make sure that they carried no lethals. Forty-one wild-type daughters (non-crossovers) were tested from such matings, to see if the non-crossover X chromosomes carried new lethals. The only lethal that occurred was in a paternal (i.e., scute echinus crossveinless cut vermilion garnet forked) chromosome. Thirty-eight double-crossover daughters and one triple-crossover (i.e., a total of 79 crossings over) were also tested; and again no lethals occurred in any of the maternally derived chromosomes, though there were two doubtful cases of new lethals in the chromosomes derived from the multiple-recessive fathers. While this experiment was done on a small scale, it furnishes no indication that crossover chromosomes are more likely to contain new lethals than are non-crossovers.

There is, however, another kind of evidence that argues against any general applicability of the unequal-crossing-over explanation of mutation, namely, the cases in which mutations

can be shown to have occurred in the X chromosomes of males, since it may be taken as established that crossing over does not occur between the X and the Y of a male.

We have seen earlier in this paper that infrabar arose from bar in a male, and that its later behavior was not in agreement with the view that it represented a quantitative change in the bar gene, as it should if due to unequal crossing over. I have also obtained yellow, a fused allelomorph, and a lozenge allelomorph under the same circumstances, namely, from mothers with attached X's and in experiments where known sex-linked genes were present, so that breaking apart of the attached X's was known not to have occurred.

Unpublished data are available for 5 other cases of the same sort, either from attached-X or from "high-non-disjunction" mothers, as follows: rudimentary (C. B. Bridges), a dusky allelomorph (C. B. Bridges), a sable allelomorph (E. M. Wallace), white (L. V. Morgan), and a new lozenge allelomorph from lozenge (C. B. Bridges). In all of these cases, as in that of infrabar, the mutant type first appeared as a single male.

Muller (1920) reported the occurrence of white as a "somatic" mutation in a male. From a stock in which white was not present he obtained a male with one wild-type eye and one white eye. This male also transmitted white to some (all that were tested) of his daughters. In the same paper Muller described briefly a mosaic male that was partly yellow, and transmitted the new character to his offspring. Doctor Bridges informs me that he has a similar (unpublished) record for yellow. Mohr (1923 a) reports a similar case for a singed allelomorph, though here some of the X-bearing sperms carried singed, while others did not. I have

observed two other such cases,—both in *D. simulans*. The mutant types dusky and fused (both corresponding to the types of the same names in *D. melanogaster*) each appeared first in an individual that showed the new character in only one wing; and in each case tests showed that some of the X-bearing sperms carried the new gene, while others did not. In all cases discussed in the last two paragraphs, genetic tests have established the allelomorphism of the new mutant genes to the old ones whose names they bear.

There is thus clear evidence that mutations have arisen in the following sex-linked loci in the germ-cells of males: yellow (3 times), white (twice), lozenge (twice), dusky (twice), fused (twice), singed (once), bar (once). It should be noted that all these loci are among the more mutable ones of *Drosophila*.

Another class of cases to which the unequal-crossing-over hypothesis is probably not applicable is that in which mutation can be shown to have occurred at some stage other than maturation. A number of such instances are on record for *Drosophila*. The mosaic males described above are examples, and a longer list of cases for autosomal mutant types and for sex-linked mutations in females could easily be compiled. But since there is evidence that crossing over does very rarely occur at somatic divisions, this evidence can hardly be considered decisive. In the case of certain types of frequently recurring somatic mutations in plants, however, the mutation occurs far too often to make an appeal to somatic crossing over seem plausible. The clearest example of this sort is the variegated pericarp of maize studied by Emerson (1917) and others, in which a given gene mutates many separate times in a single individual plant.

Mutations are known in which there appeared to be no crossing over in the region concerned,—both in females and in males where crossing over does not normally occur at all. The previously cited cases of mutations in the X chromosomes of males are examples. These may seem to furnish conclusive evidence that mutation need not be accompanied by crossing over. There is, however, one possibility that needs to be considered in this connection.

Recent results (not yet published, but soon to appear) obtained with triploid females (Bridges and Anderson) and with females having unlike attached X's (Anderson, L. V. Morgan, and Sturtevant) have shown that crossing over must normally occur when the homologous chromosomes are doubled: that is, in a "four-strand stage" (in diploid females). These results show also that crossing over may occur between only two of the strands at a given level. Now, if it be supposed that *sister* strands may cross over with each other, there will result chromosomes in which no rearrangement of mutant genes has occurred, since sister strands come from the division of one chromosome and will be identical in the genes that they carry. Yet it is conceivable that such crossing over might be unequal, and in such a case might lead to the production of a new mutation that did not appear to be due to crossing over.

The data presented in this paper show that such an event must be extremely rare in the case of bar, since no clear case was found of bar mutation (in a female) unaccompanied by evident crossing over between forked and fused. The few exceptional cases may be accounted for in this way; but, as pointed out when they were described, it seems at least equally probable that all of them are due to experimental errors. We must conclude that

sister strands do not cross over with each other; or, if they do, that the crossing over is rarely, if ever, unequal.

It is therefore unlikely that apparent non-crossover mutations in other loci are to be referred to crossing over between sister strands.

"PRESENCE AND ABSENCE" AND QUANTITATIVE VIEW OF MUTATION

It will be observed that the hypothesis advocated in this paper makes bar, double-bar and round by reversion (or infrabar, double-infrabar and round by reversion) represent quantitative variations of the same substance. In the case of bar and round, the hypothesis is the same as the original and most special type of quantitative view, the "presence and absence" hypothesis. But the present scheme differs from the earlier ones in that it is based on definite evidence for the occurrence of unequal crossing over. That is, the mechanism whereby the quantitative differences are brought about is an essential part of the hypothesis. In the preceding section we have seen that there is definite evidence to show that unequal crossing over is not usual in the production of new mutant types. It is especially noteworthy that this evidence was derived in part from the white locus of *Drosophila* and the variegated locus of maize,—two of the best-known examples of loci that have produced large series of multiple allelomorphs. It is clear, therefore, that the bar case does not furnish support to the idea that mutations in general are quantitative in nature. Even with respect to multiple allelomorphs, where the quantitative view has often been urged, it is obvious that, at least in the cases of white and variegated, the bar evidence does not in any way support that view.

ARE DEFICIENCIES DUE TO UNEQUAL
CROSSING OVER?

The "section-deficiencies" described by Bridges (1917, 1919) and by Mohr (1919, 1923 b) are probably to be interpreted as due to losses of definite sections of chromosomes. It will be observed that bar reversion has here been treated as due to the loss of a very short section; it may accordingly be described as a deficiency that is too short to show the lethal effect and other properties of the previously described deficiencies. When the case is stated in this way, the question at once arises: is it probable that notch and other deficiencies have also arisen through unequal crossing over? If so, the contrary crossover should be a chromosome that was double for a region corresponding to the deficient section. Such a chromosome has never been identified, but it may be doubted if it would be detected even if present. Furthermore, it might well be lethal even in heterozygous females, in which case it would not be capable of detection.

There is evidence that deficiencies may arise in other ways than by unequal crossing over. In at least one case (Bridges and Morgan 1923) the section missing from a second chromosome was found to be present, but attached to a third chromosome. In this case, then, the deficiency can not have been due to unequal crossing over. The first deficiency described, that for forked and bar (Bridges 1917), occurred first as a single female that had obtained the deficient X from her father. Here the deficiency arose (either in a male or very early in the cleavage of a female zygote) at a time when crossing over (and bar reversion) does not normally occur. In the case of notch, also, there is evidence that the deficiency may originate at stages other than maturation. Lance-

field (1922) records the occurrence of a notch (probably corresponding to that of *D. melanogaster*) in *Drosophila obscura*; the mutation was first detected as two females from a pair mating that gave numerous offspring. In this case the deficiency must have originated in the gonial cells of one parent, unless the two notch females received their notch chromosomes from the father, in which case it is just possible that they came from two sperms derived from a single spermatocyte. But in this case the hypothesis of unequal crossing over remains as improbable as before. I have observed two cases in *D. melanogaster* that represent "somatic" (i.e., not occurring at the maturation divisions) occurrences of notch. In one case three notch females were produced from a single mother. The X's of the mother were attached, and the notch daughters, like all their sisters, did not carry a paternal X. These three females were all sterile, so here it was not possible to demonstrate that the new type was actually notch; but the numerous characters of notch make the identification very probable. The other case also occurred in a line in which the females all had attached X's. A female, from a line with no notch ancestry, was notch in the left wing but not in the right. The offspring showed that this female was, like her mother, heterozygous for several sex-linked genes. These included scute, 3 units to the left of notch, and crossveinless, 10 units to the right of it. Some of the eggs of the mosaic carried notch, but many of them did not. Furthermore, tests showed that scute and crossveinless were in opposite chromosomes in both types of eggs; that is, the mutation to notch occurred at a cleavage division, and was not accompanied by crossing over between scute and crossveinless. From these three instances we may conclude that

the notch deficiency may arise at stages in the life cycle at which crossing over and bar reversion do not normally occur, and, in the third case, there is definite evidence that crossing over did not occur. While it may still be supposed that unequal crossing over will sometimes give rise to section deficiencies, the evidence indicates that the three best-known examples of section deficiencies in *Drosophila* have not arisen in that way.

UNEQUAL CROSSING OVER AND THE EXACT NATURE OF SYNAPSIS

The data on crossing over have all indicated consistently that when two chromosomes cross over they do so at exactly corresponding levels. The case of bar is the first one in which any inequality of crossing-over levels has been detected; and we have seen in the preceding sections that an analysis of other possible instances of such an occurrence makes it probable that they must be explained in some other way. The case of bar is clearly quite exceptional. But it does serve to suggest that the exact correspondence of crossover levels, that is so constant, is not to be referred to a property common to all the genes. For unequal crossing over occurs in females that are homozygous for bar or for infrabar, and in such females these loci are alike in the two X chromosomes that cross over unequally. It is difficult to imagine how the chromosomes can pair so extremely exactly as they must do, unless in some way like genes come to lie side by side. But the present case indicates that this interpretation will have to be applied with some caution.

SUMMARY

1. Sixteen different kinds of changes at the bar locus are shown to occur exclusively, or nearly so, in eggs that undergo crossing over at or near the bar locus.

2. This result can be explained if it is supposed that such crossovers are unequal, so that one daughter chromosome gets two representatives of the bar locus while the other receives none.

3. Only one mutation in this locus has been shown to have occurred in the germ track of a male. This one gave rise (from bar) to a new and less extreme allelomorph called infrabar.

4. Infrabar does not appear to represent a quantitative change in the bar gene.

5. When, by unequal crossing over, bar and infrabar come to lie in the same chromosome, they maintain their separate identities, and may be recovered again.

6. In such double forms the two elements also maintain their sequence in the same linear series as the rest of the genes. It is thus possible to obtain bar-infrabar and also infrabar-bar. These two types look alike, but can be distinguished by their origin and by the usual tests for determining the sequence of genes.

7. Facet counts are given for all the possible combinations of the following members of the bar series: round, infrabar, bar, double-infrabar, bar-infrabar, double-bar.

8. Analysis of these data shows that two genes lying in the same chromosome are more effective on development than are the same two genes when they lie in different chromosomes.

9. A general survey makes it seem improbable that many mutations in other loci are to be explained as due to unequal crossing over.

LITERATURE CITED

- Bridges, C. B. 1917 Deficiency. *Genetics* 2: 445-465.
 1919 Vermilion deficiency. *Jour. Gen. Physiol.* 1: 645-656.
 1921 Triploid intersexes in *Drosophila melanogaster*. *Science*, n.s. 54: 252-254.

- Bridges, C. B., and Morgan, T. H. 1923 The third chromosome group of mutant characters of *Drosophila melanogaster*. *Carnegie Inst. Washington Publ.* 327, 251 pp.
- Emerson, R. A. 1917 Genetical studies of variegated pericarp in maize. *Genetics* 2: 1-35.
- Hersh, A. H. 1924 The effect of temperature upon the heterozygotes in the bar series of *Drosophila*. *Jour. Exper. Zoöl.* 39: 55-71.
- Hersh, R. K. 1924 The effect of temperature upon the full-eyed race of *Drosophila*. *Jour. Exper. Zoöl.* 39: 43-53.
- Krafka, J. 1920 The effect of temperature upon facet number in the bar-eyed mutant of *Drosophila*. *Jour. Gen. Physiol.* 2: 409-464.
- Lancefield, D. E. 1922 Linkage relations of the sex-linked characters of *Drosophila obscura*. *Genetics* 4: 335-384.
- May, H. G. 1917 Selection for higher and lower facet numbers in the bar-eyed race of *Drosophila* and the appearance of reverse mutations. *Biol. Bull.* 33: 361-395.
- Metz, C. W. 1916 Chromosome studies in the Diptera. II. The paired association of chromosomes in the Diptera and its significance. *Jour. Exper. Zoöl.* 21: 213-279.
- Mohr, O. L. 1919 Character changes caused by mutation of an entire region of a chromosome in *Drosophila*. *Genetics* 4: 275-282.
- 1923 a A somatic mutation in the singed locus of the X chromosome of *Drosophila melanogaster*. *Hereditas* 4: 142-160.
- 1923 b A genetic and cytological analysis of a section deficiency involving four units of the X chromosome in *Drosophila melanogaster*. *Zeitschr. indukt. Abstammn. u. Vererb.* 32: 108-232.
- Morgan, L. V. 1922 Non-criss-cross inheritance in *Drosophila melanogaster*. *Biol. Bull.* 42: 267-274.
- Morgan, T. H., and Bridges, C. B. 1916 Sex-linked inheritance in *Drosophila*. *Carnegie Inst. Washington Publ.* 237, 87 pp. 2pls.
- Muller, H. J. 1920 Further changes in the white-eye series of *Drosophila* and their bearing on the manner of occurrence of mutation. *Jour. Exper. Zoöl.* 31: 443-473.
- Seyster, E. W. 1919 Eye facet number as influenced by temperature in the bar-eyed mutant of *Drosophila melanogaster*. *Biol. Bull.* 37: 168-182.
- Sturtevant, A. H., and Morgan, T. H. 1923 Reverse mutation of the bar gene correlated with crossing over. *Science*, n.s. 57: 746-747.
- Tice, S. C. 1914 A new sex-linked character in *Drosophila*. *Biol. Bull.* 26: 221-230.
- Weinstein, A. 1918 Coincidence of crossing over in *Drosophila melanogaster*. *Genetics* 3: 135-173.
- Zeleny, C. 1919 A change in the bar gene of *Drosophila* involving further decrease in facet number and increase in dominance, *Jour. Gen. Physiol.* 2: 69-71.
- 1921 The direction and frequency of mutation in the bar-eye series of multiple allelomorphs of *Drosophila*. *Jour. Exper. Zoöl.* 34: 203-233.
- 1922 The effect of selection for eye facet number in the white bar-eye race of *Drosophila melanogaster*. *Genetics* 7: 1-115.
- 1923 The temperature coefficient of a heterozygote with an expression for the value of a germinal difference in terms of an environmental one. *Biol. Bull.* 44: 105-112.
- Zeleny, C., and Mattoon, E. W. 1915 The effect of selection upon the "bar-eye" mutant of *Drosophila*. *Jour. Exper. Zoöl.* 19: 515-529.



Artificial Transmutation of the Gene

H. J. MULLER

Reprinted by publisher's permission from *Science*, vol. 66, 1927, pp. 84-87.

One of the major turning points in the study of heredity was the discovery that changes in the genes could be induced by artificial means. This short paper by Muller describes the first demonstrably successful attempt to make genes mutate, and represents the beginning of a new epoch in genetic history. Mutagenic methods and materials are now well known and widely used in genetic experiments, and probably represent the most useful known tool in research. As a consequence of this paper, the X-ray machine has become as common a piece of equipment in a genetics laboratory as is the microscope in histology.

It should be noted that Muller recognizes the dangers of X-ray treatment to germinal tissues in this, the first paper on induced mutations. Muller's recognition of the dual nature of the effects of irradiation is clear cut, for he points out in a paragraph on page 152 that first there is a direct effect on the cells and tissues, and second there is an indirect effect on the descendant cells and tissues through the mediation of the genes and chromosomes. The first of these is transient (although possibly fatal), while the second is permanent. It is an interesting commentary that there is still active and quite acrimonious debate today as to the seriousness and consequences of this and other kinds of irradiation damage.

MOST MODERN GENETICISTS WILL agree that gene mutations form the chief basis of organic evolution, and therefore of most of the complexities of living things. Unfortunately for the geneticists, however, the study of these mutations, and, through them, of the genes themselves, has heretofore been very seriously hampered by the extreme infrequency of their occurrence under ordinary conditions, and by the general unsuccessfulness of attempts to

modify decidedly, and in a sure and detectable way, this sluggish "natural" mutation rate. Modification of the innate nature of organisms, for more directly utilitarian purposes, has of course been subject to these same restrictions, and the practical breeder has hence been compelled to remain content with the mere making of recombinations of the material already at hand, providentially supplemented, on rare and isolated occasions, by an un-

expected mutational windfall. To these circumstances are due the wide-spread desire on the part of biologists to gain some measure of control over the hereditary changes within the genes.

It has been repeatedly reported that germinal changes, presumably mutational, could be induced by X or radium rays, but, as in the case of the similarly published claims involving other agents (alcohol, lead, antibodies, etc.), the work has been done in such a way that the meaning of the data, as analyzed from a modern genetic standpoint, has been highly disputatious at best; moreover, what were apparently the clearest cases have given negative or contrary results on repetition. Nevertheless, on theoretical grounds, it has appeared to the present writer that radiations of short wave length should be especially promising for the production of mutational changes, and for this and other reasons a series of experiments concerned with this problem has been undertaken during the past year on the fruit fly, *Drosophila melanogaster*, in an attempt to provide critical data. The well-known favorableness of this species for genetic study, and the special methods evolved during the writer's eight years' intensive work on its mutation rate (including the work on temperature, to be referred to later), have finally made possible the finding of some decisive effects, consequent upon the application of X-rays. The effects here referred to are truly mutational, and not to be confused with the well-known effects of X-rays upon the distribution of the chromatin, expressed by non-disjunction, non-inherited crossover modifications, etc. In the present condensed digest of the work, only the broad facts and conclusions therefrom, and some of the problems raised, can be presented, without any

details of the genetic methods employed, or of the individual results obtained.

It has been found quite conclusively that treatment of the sperm with relatively heavy doses of X-rays induces the occurrence of true "gene mutations" in a high proportion of the treated germ cells. Several hundred mutants have been obtained in this way in a short time and considerably more than a hundred of the mutant genes have been followed through three, four or more generations. They are (nearly all of them, at any rate) stable in their inheritance, and most of them behave in the manner typical of the Mendelian chromosomal mutant genes found in organisms generally. The nature of the crosses was such as to be much more favorable for the detection of mutations in the X-chromosomes than in the other chromosomes, so that most of the mutant genes dealt with were sex-linked; there was, however, ample proof that mutations were occurring similarly throughout the chromatin. When the heaviest treatment was given to the sperm, about a seventh of the offspring that hatched from them and bred contained individually detectable mutations in their treated X-chromosome. Since the X forms about one fourth of the haploid chromatin, then, if we assume an equal rate of mutation in all the chromosomes (per unit of their length), it follows that almost "every other one" of the sperm cells capable of producing a fertile adult contained an "individually detectable" mutation in some chromosome or other. Thousands of untreated parent flies were bred as controls in the same way as the treated ones. Comparison of the mutation rates under the two sets of conditions showed that the heavy treatment had caused a rise of about

fifteen thousand per cent. in the mutation rate over that in the untreated germ cells.

Regarding the types of mutations produced, it was found that, as was to have been expected both on theoretical grounds and on the basis of the previous mutation studies of Altenburg and the writer, the lethals (recessive for the lethal effect, though some were dominant for visible effects) greatly outnumbered the non-lethals producing a visible morphological abnormality. There were some "semi-lethals" also (defining these as mutants having a viability ordinarily between about 0.5 per cent. and 10 per cent. of the normal), but, fortunately for the use of lethals as an index of mutation rate, these were not nearly so numerous as the lethals. The elusive class of "invisible" mutations that caused an even lesser reduction of viability, not readily confusable with lethals, appeared larger than that of the semi-lethals, but they were not subjected to study. In addition, it was also possible to obtain evidence in these experiments for the first time, of the occurrence of dominant lethal genetic changes, both in the X and in the other chromosomes. Since the zygotes receiving these never developed to maturity, such lethals could not be detected individually, but their number was so great that through egg counts and effects on the sex ratio evidence could be obtained of them *en masse*. It was found that their numbers are of the same order of magnitude as those of the recessive lethals. The "partial sterility" of treated males is, to an appreciable extent at least, caused by these dominant lethals. Another abundant class of mutations not previously recognized was found to be those which, when heterozygous, cause sterility but produce no detectable

change in appearance; these too occur in numbers rather similar to those of the recessive lethals, and they may hereafter afford one of the readiest indices of the general mutation rate, when this is high. The sterility thus caused, occurring as it does in the offspring of the treated individuals, is of course a separate phenomenon from the "partial sterility" of the treated individuals themselves, caused by the dominant lethals.

In the statement that the proportion of "individually detectable mutations" was about one seventh for the X, and therefore nearly one half for all the chromatin, only the recessive lethals and semi-lethals and the "visible" mutants were referred to. If the dominant lethals, the dominant and recessive sterility genes and the "invisible" genes that merely reduce (or otherwise affect) viability or fertility had been taken into account, the percentage of mutants given would have been far higher, and it is accordingly evident that in reality the great majority of the treated sperm cells contained mutations of some kind or other. It appears that the rate of gene mutation after X-ray treatment is high enough, in proportion to the total number of genes, so that it will be practicable to study it even in the case of individual loci, in an attack on problems of allelomorphism, etc.

Returning to a consideration of the induced mutations that produced visible effects, it is to be noted that the conditions of the present experiment allowed the detection of many which approached or overlapped the normal type to such an extent that ordinarily they would have escaped observation, and definite evidence was thus obtained of the relatively high frequency of such changes here, as compared with the more conspicuous ones. The

belief has several times been expressed in the *Drosophila* literature that this holds true in the case of "natural" mutations in this organism, but it has been founded only on "general impressions"; Baur, however, has demonstrated the truth of it in *Antirrhinum*. On the whole, the visible mutations caused by raying were found to be similar, in their general characteristics, to those previously detected in non-rayed material in the extensive observations on visible mutations in *Drosophila* carried out by Bridges and others. A considerable proportion of the induced visible mutations were, it is true, in loci in which mutation apparently had never been observed before, and some of these involved morphological effects of a sort not exactly like any seen previously (e.g., "splotted wing," "sex-combless," etc.), but, on the other hand, there were also numerous repetitions of mutations previously known. In fact, the majority of the well-known mutations in the X-chromosome of *Drosophila melanogaster*, such as "white-eye," "miniature wing," "forked bristles," etc., were reobtained, some of them several times. Among the visible mutations found, the great majority were recessive, yet there was a considerable "sprinkling" of dominants, just as in other work. All in all, then, there can be no doubt that many, at least, of the changes produced by X-rays are of just the same kind as the "gene mutations" which are obtained, with so much greater rarity, without such treatment, and which we believe furnish the building blocks of evolution.

In addition to the gene mutations, it was found that there is also caused by X-ray treatment a high proportion of rearrangements in the linear order of the genes. This was evidenced in general by the frequent inherited disturbances in crossover frequency (at least

3 per cent. were detected in the X-chromosome alone, many accompanied but some unaccompanied by lethal effects), and evidenced specifically by various cases that were proved in other ways to involve inversions, "deficiencies," fragmentations, translocations, etc., of portions of a chromosome. These cases are making possible attacks on a number of genetic problems otherwise difficult of approach.

The transmitting action of X-rays on the genes is not confined to the sperm cells, for treatment of the unfertilized females causes mutations about as readily as treatment of the males. The effect is produced both on oöcytes and early oögonia. It should be noted especially that, as in mammals, X-rays (in the doses used) cause a period of extreme infertility, which commences soon after treatment and later is partially recovered from. It can be stated positively that the return of fertility does not mean that the new crop of eggs is unaffected, for these, like those mature eggs that managed to survive, were found in the present experiments to contain a high proportion of mutant genes (chiefly lethals, as usual). The practice, common in current X-ray therapy, of giving treatments that do not certainly result in permanent sterilization, has been defended chiefly on the ground of a purely theoretical conception that eggs produced after the return of fertility must necessarily represent "uninjured" tissue. As this presumption is hereby demonstrated to be faulty it would seem incumbent for medical practice to be modified accordingly, at least until genetically sound experimentation upon mammals can be shown to yield results of a decisively negative character. Such work upon mammals would involve a highly elaborate undertaking, as compared with the above experiments on flies.

From the standpoint of biological theory, the chief interest of the present experiments lies in their bearing on the problems of the composition and behavior of chromosomes and genes. Through special genetic methods it has been possible to obtain some information concerning the manner of distribution of the transmuted genes amongst the cells of the first and later zygote generations following treatment. It is found that the mutation does not usually involve a permanent alteration of all of the gene substance present at a given chromosome locus at the time of treatment, but either affects in this way only a portion of that substance, or else occurs subsequently, as an after-effect, in only one of two or more descendant genes derived from the treated gene. An extensive series of experiments, now in project, will be necessary for deciding conclusively between these two possibilities, but such evidence as is already at hand speaks rather in favor of the former. This would imply a somewhat compound structure for the gene (or chromosome as a whole) in the sperm cell. On the other hand, the mutated tissue is distributed in a manner that seems inconsistent with a general applicability of the theory of "gene elements" first suggested by Anderson in connection with variegated pericarp in maize, then taken up by Eyster, and recently reenforced by Demerec in *Drosophila virilis*.

A precociously doubled (or further multiplied) condition of the chromosomes (in "preparation" for later mitoses) is all that is necessary to account for the above-mentioned *fractional effect* of X-rays on a given locus; but the theory of a divided condition of each gene, into a number of (originally identical) "elements" that can become separated somewhat indeterminately at mitosis, would lead

to expectations different from the results that have been obtained in the present work. It should, on that theory, often have been found here, as in the variegated corn and the ever-sporting races of *D. virilis*, that mutated tissue gives rise to normal by frequent "reverse mutation"; moreover, treated tissues not at first showing a mutation might frequently give rise to one, through a "sorting out" of diverse elements, several generations after treatment. Neither of these effects was found. As has been mentioned, the mutants were found to be stable through several generations, in the great majority of cases at least. Hundreds of non-mutated descendants of treated germ cells, also, were carried through several generations, without evidence appearing of the production of mutations in generations subsequent to the first. Larger numbers will be desirable here, however, and further experiments of a different type have also been planned in the attack on this problem of gene structure, which probably can be answered definitely.

Certain of the above points which have already been determined, especially that of the fractional effect of X-rays, taken in conjunction with that of the production of dominant lethals, seem to give a clue to the especially destructive action of X-rays on tissues in which, as in cancer, embryonic and epidermal tissues, the cells undergo repeated divisions (though the operation of additional factors, e.g., abnormal mitoses, tending towards the same result, is not thereby precluded); moreover, the converse effect of X-rays, in occasionally producing cancer, may also be associated with their action in producing mutations. It would be premature, however, at this time to consider in detail the various X-ray effects previously considered as "physiological," which may

now receive a possible interpretation in terms of the gene-transmuting property of X-rays; we may more appropriately confine ourselves here to matters which can more strictly be demonstrated to be genetic.

Further facts concerning the nature of the gene may emerge from a study of the comparative effects of varied dosages of X-rays, and of X-rays administered at different points in the life cycle and under varied conditions. In the experiments herein reported, several different dosages were made use of, and while the figures are not yet quite conclusive they make it probable that, within the limits used, the number of recessive lethals does not vary directly with the X-ray energy absorbed, but more nearly with the square root of the latter. Should this lack of exact proportionality be confirmed, then, as Dr. Irving Langmuir has pointed out to me, we should have to conclude that these mutations are not caused directly by single quanta of X-ray energy that happen to be absorbed at some critical spot. If the transmuting effect were thus relatively indirect there would be a greater likelihood of its being influenceable by other physico-chemical agencies as well, but our problems would tend to become more complicated. There is, however, some danger in using the total of lethal mutations produced by X-rays as an index of gene mutations occurring in single loci, for some lethals, involving changes in crossover frequency, are probably associated with rearrangements of chromosome regions, and such changes would be much less likely than "point mutations" to depend on single quanta. A re-examination of the effect of different dosages must therefore be carried out, in which the different types of mutations are clearly distinguished from one another. When

this question is settled, for a wide range of dosages and developmental stages, we shall also be in a position to decide whether or not the minute amounts of gamma radiation present in nature cause the ordinary mutations which occur in wild and in cultivated organisms in the absence of artificially administered X-ray treatment.

As a beginning in the study of the effect of varying other conditions, upon the frequency of the mutations produced by X-rays, a comparison has been made between the mutation frequencies following the raying of sperm in the male and in the female receptacles, and from germ cells that were in different portions of the male genital system at the time of raying. No decisive differences have been observed. It is found, in addition, that aging the sperm after treatment, before fertilization, causes no noticeable alteration in the frequency of detectable mutations. Therefore the death rate of the mutant sperm is no higher than that of the unaffected ones; moreover, the mutations can not be regarded as secondary effects of any semi-lethal physiological changes which might be supposed to have occurred more intensely in some ("more highly susceptible") spermatozoa than in others.

Despite the "negative results" just mentioned, however, it is already certain that differences in X-ray influences, by themselves, are not sufficient to account for all variations in mutation frequency, for the present X-ray work comes on the heels of the determination of mutation rate being dependent upon temperature (work as yet unpublished). This relation had first been made probable by work of Altenburg and the writer in 1918, but was not finally established until the completion of some experiments in 1926. These gave the first definite evi-

dence that gene mutation may be to any extent controllable, but the magnitude of the heat effect, being similar to that found for chemical reactions in general, is too small, in connection with the almost imperceptible "natural" mutation rate, for it, by itself, to provide a powerful tool in the mutation study. The result, however, is enough to indicate that various factors besides X-rays probably do affect the composition of the gene, and that the measurement of their effects, at least when in combination with X-rays, will be practicable. Thus we may hope that problems of the composition and behavior of the gene can shortly be approached from various new angles, and new handles found for their investigation, so that it will be legitimate to speak of the subject of "gene physiology," at least, if not of gene physics and chemistry.

In conclusion, the attention of those working along classical genetic lines may be drawn to the opportunity,

afforded them by the use of X-rays, of creating in their chosen organisms a series of artificial races for use in the study of genetic and "phaenogenetic" phenomena. If, as seems likely on general considerations, the effect is common to most organisms, it should be possible to produce, "to order," enough mutations to furnish respectable genetic maps, in their selected species, and by the use of the mapped genes, to analyze the aberrant chromosome phenomena simultaneously obtained. Similarly, for the practical breeder, it is hoped that the method will ultimately prove useful. The time is not ripe to discuss here such possibilities with reference to the human species.

The writer takes pleasure in acknowledging his sincere appreciation of the cooperation of Dr. Dalton Richardson, Roentgenologist, of Austin, Texas, in the work of administering the X-ray treatments.



A Correlation of Cytological and Genetical Crossing-OVER in *Zea mays*

HARRIET B. CREIGHTON and BARBARA MCCLINTOCK

Reprinted by authors' and publisher's permission from *Proceedings of the National Academy of Sciences*, vol. 17, 1931, pp. 492-497.

The Sutton-Boveri Hypothesis (page 27) has been successively strengthened by several of the papers in this collection. First Bateson and Punnett unknowingly demonstrated linkage, a phenomenon best

explained by the hypothesis. Then Morgan showed that a specific locus could be assigned to a specific chromosome. Sturtevant's demonstration of a linear arrangement of genes added another link to the proof, and his work on bar-eye also substantiated the hypothesis (how completely will be seen in Bridges' paper on page 163). Now we come to an analysis that puts the final link in the chain, for here we see correlations between cytological evidence and genetic results that are so strong and obvious that their validity cannot be denied.

This paper has been called a landmark in experimental genetics. It is more than that—it is a cornerstone. It is not an easy paper to follow, for the items that require retention throughout the analysis are many, and it is fatal to one's understanding to lose track of any of them. Mastery of this paper, however, can give one the strong feeling of being able to master almost anything else he might have to wrestle with in biology.

A REQUIREMENT FOR THE GENETICAL study of crossing-over is the heterozygous condition of two allelomorphic factors in the same linkage group. The analysis of the behavior of homologous or partially homologous chromosomes, which are morphologically distinguishable at two points, should show evidence of cytological crossing-over. It is the aim of the present paper to show that cytological crossing-over occurs and that it is accompanied by genetical crossing-over.

In a certain strain of maize the second-smallest chromosome (chromosome 9) possesses a conspicuous knob at the end of the short arm. Its distribution through successive generations is similar to that of a gene. If a plant possessing knobs at the ends of both of its 2nd-smallest chromosomes is crossed to a plant with no knobs, cytological observations show that in the resulting F_1 individuals only one member of the homologous pair possesses a knob. When such an individual is back-crossed to one having no knob on either chromosome, half of the offspring are heterozygous for the knob and half possess no knob at all. The knob, therefore, is a constant feature of the chromosome possessing it. When present on one chromosome and

not on its homologue, the knob renders the chromosome pair visibly heteromorphic.

In a previous report¹ it was shown that in a certain strain of maize an interchange had taken place between chromosome 8 and 9. The interchanged pieces were unequal in size; the long arm of chromosome 9 was increased in relative length, whereas the long arm of chromosome 8 was correspondingly shortened. When a gamete possessing these two interchanged chromosomes meets a gamete containing a normal chromosome set, meiosis in the resulting individual is characterized by a side-by-side synapsis of homologous parts. Therefore, it should be possible to have crossing-over between the knob and the interchange point.

In the previous report it was also shown that in such an individual the only functioning gametes are those which possess either the two normal chromosomes (N, n) or the two interchanged chromosome (I, i), i.e., the full genome in one or the other arrangement. The functional gametes therefore possess either the shorter,

¹ McClintock, B., *Proc. Nat. Acad. Sci.*, 16:791-796, 1930.

normal, knobbed chromosome (*n*) or the longer, interchanged, knobbed chromosome (*I*). Hence, when such a plant is crossed to a plant possessing the normal chromosome complement, the presence of the normal chromosome in functioning gametes of the former will be indicated by the appearance of ten bivalents in the prophase of meiosis of the resulting individuals. The presence of the interchanged chromosome in other gametes will be indicated in other *F*₁ individuals by the appearance of eight bivalents plus a ring of four chromosomes in the late prophase of meiosis.

If a gamete possessing a normal chromosome number 9 with no knob, meets a gamete possessing an interchanged chromosome with a knob, it is clear that these two chromosomes which synapse along their homologous parts during prophase of meiosis in the resulting individual are visibly different at each of their two ends. If no crossing-over occurs, the gametes formed by such an individual will contain either the knobbed, interchanged chromosome (*a*, Fig. 1) or the normal chromosome without a knob (*d*, Fig. 1). Gametes containing either a knobbed, normal chromosome (*c*, Fig. 1) or a knobless, interchanged chromosome (*b*, Fig. 1) will be formed as a result of crossing-over. If such an individual is crossed to a plant possessing two normal knobless chromosomes, the resulting individuals will be of four kinds. The non-crossover gametes would give rise to individuals which show either (1) ten bivalents at prophase of meiosis and no knob on chromosome 9, indicating that a gamete with a chromosome of type *d* has functioned or (2) a ring of four chromosomes with a single conspicuous knob, indicating that a gamete of type *a* has functioned. The crossover types will be recognizable as individuals which possess either (1) ten bi-

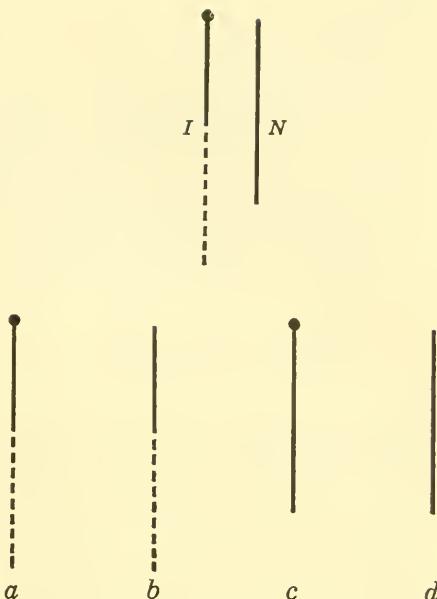


Fig. 1. (above) Diagram of the chromosomes in which crossing over was studied.

(below) Diagram of chromosome types found in gametes of a plant with the constitution shown above.

a—Knobbed, interchanged chromosome

b—Knobless, interchanged chromosome

c—Knobbed, normal chromosome

d—Knobless, normal chromosome

a and *d* are non-crossover types.

b and *c* are crossover types.

valents and a single knob associated with bivalent chromosome 9 or (2) a ring of four chromosomes with no knob, indicating that crossover gametes of types *c* and *b*, respectively, have functioned. The results of such a cross are given in culture 337, table 1. Similarly, if such a plant is crossed to a normal plant possessing knobs at the ends of both number 9 chromosomes and if crossing-over occurs, the resulting individuals should be of four kinds. The non-crossover types would be represented by (1) plants homozygous for the knob and possessing the interchanged chromosome and (2)

TABLE 1

<u>Knob-interchanged</u> Knobless-normal		Knobless-normal, culture 337 and Knobbed-normal cultures A125 and 340			
Culture		Plants possessing 2 normal chromosomes		Plants possessing an interchanged chromosome	
		Non-crossovers	Crossovers	Non-crossovers	Crossovers
337		8	3	6	2
A125		39	31	36	23
340		5	3	5	3
Totals		52	37	47	28

plants heterozygous for the knob and possessing two normal chromosomes. The functioning of gametes which had been produced as the result of crossing-over between the knob and the interchange would give rise to (1) individuals heterozygous for the knob and possessing the interchanged chromosome and (2) those homozygous for the knob and possessing two normal chromosomes. The results of such crosses are given in culture A125 and 340, table 1. Although the data are few, they are consistent. The amount of crossing-over between the knob and the interchange, as measured from these data, is approximately 39%.

In the preceding paper it was shown that the knobbed chromosome carries the genes for colored aleurone (*C*), shrunken endosperm (*sh*) and waxy endosperm (*wx*). Furthermore, it was shown that the order of these genes, beginning at the interchange point is *wx-sh-c*. It is possible, also, that these genes all lie in the short arm of the

knobbed chromosome. Therefore, a linkage between the knob and these genes is to be expected.

One chromosome number 9 in a plant possessing the normal complement had a knob and carried the genes *C* and *wx*. Its homologue was knobless and carried the genes *c* and *Wx*. The non-crossover gametes should contain a knobbed-*C-wx* or a knobless-*c-Wx* chromosome. Crossing-over in region 1 (between the knob and *C*) would give rise to knobless-*C-wx* and knobbed-*c-Wx* chromosomes. Crossing-over in region 2 (between *C* and *wx*) would give rise to knobbed-*C-Wx* and knobless-*c-wx* chromosomes. The results of crossing such a plant to a knobless-*c-wx* type are given in table 2. It should be expected on the basis of interference that the knob and *C* would remain together when a crossover occurred between *C* and *wx*; hence, the individuals arising from colored starchy (*C-Wx*) kernels should possess a knob, whereas those

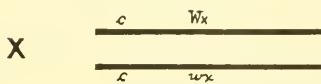
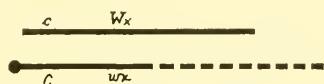
TABLE 2

<u>Knob-<i>C-wx</i></u> Knobless- <i>c-Wx</i>		Knobless- <i>c-wx</i>					
<i>C-wx</i>		<i>c-Wx</i>		<i>C-Wx</i>		<i>c-wx</i>	
Knob	Knobless	Knob	Knobless	Knob	Knobless	Knob	Knobless
12	5	5	34	4	0	0	3

coming from colorless, waxy (*c-wx*) kernels should be knobless. Although the data are few they are convincing. It is obvious that there is a fairly close association between the knob and *C*.

To obtain a correlation between cytological and genetic crossing-over it is necessary to have a plant heteromorphic for the knob, the genes *c* and *wx* and the interchange. Plant 338

(17) possessed in one chromosome the knob, the genes *C* and *wx* and the interchanged piece of chromosome 8. The other chromosome was normal, knobless and contained the genes *c* and *Wx*. This plant was crossed to an individual possessing two normal, knobless chromosomes with the genes *c-Wx* and *c-wx*, respectively. This cross is diagrammed as follows:



The results of the cross are given in table 3. In this case all the colored kernels gave rise to individuals possess-

ing a knob, whereas all the colorless kernels gave rise to individuals showing no knob.

TABLE 3

		Knob-C-wx-interchanged Knobless-c-Wx-normal	\times	Knobless-c-Wx-normal Knobless-c-wx-normal
Plant number	Knobbed or Knobless	Interchanged or normal		
Class I, C-wx kernels				
1	Knob	Interchanged		
2	Knob	Interchanged		
3	Knob	Interchanged		
Class II, c-wx kernels				
1	Knobless	Interchanged		
2	Knobless	Interchanged		
Class III, C-Wx kernels				Pollen
1	Knob	Normal		WxWx
2	Knob	Normal	
3	Normal		WxWx
5	Knob	Normal	
6	Knob
7	Knob	Normal	
8	Knob	Normal	
Class IV, c-Wx kernels				
1	Knobless	Normal		Wxxw
2	Knobless	Normal		Wxxw
3	Knobless	Interchanged		Wxxw
4	Knobless	Normal		Wxxw
5	Knobless	Interchanged		WxWx
6	Knobless	Normal		WxWx
7	Knobless	Interchanged		Wxxw
8	Knobless	Interchanged		WxWx
9	Knobless	Normal		WxWx
10	Knobless	Normal		WxWx
11	Knobless	Normal		Wxxw
12	Knobless	Normal		Wxxw
13	Knobless	Normal		WxWx
14	Knobless	Normal		WxWx
15	Knobless	Normal		Wx...

The amount of crossing-over between the knob and the interchange point is approximately 39% (table 1), between *c* and the interchange approximately 33%, between *wx* and the interchange, 13% (preceding paper). With this information in mind it is possible to analyze the data given in table 3. The data are necessarily few since the ear contained but few kernels. The three individuals in class I are clearly non-crossover types. In class II the individuals have resulted from a crossover in region 2, i.e., between *c* and *wx*. In this case a crossover in region 2 has not been accompanied by a crossover in region 1 (between the knob and *C*) or region 3 (between *wx* and the interchange). All the individuals in class III had normal chromosomes. Unfortunately, pollen was obtained from only 1 of the 6 individuals examined for the presence of the knob. This one individual was clearly of the type expected to come from a gamete produced through crossing-over in region 2. Class IV is more difficult to analyze. Plants 6, 9, 10, 13, and 14 are normal and *WxWx*;

they therefore represent non-cross-over types. An equal number of non-crossover types are expected among the normal *Wxwx* class. Plants 1, 2, 4, 11 and 12 may be of this type. It is possible but improbable that they have arisen through the union of a *c-Wx* gamete with a gamete resulting from a double crossover in region 2 and 3. Plants 5 and 8 are single crossovers in region 3, whereas plants 3 and 7 probably represent single crossovers in region 2 or 3.

The foregoing evidence points to the fact that cytological crossing-over occurs and is accompanied by the expected types of genetic crossing-over.

Conclusions.—Pairing chromosomes, heteromorphic in two regions, have been shown to exchange parts at the same time they exchange genes assigned to these regions.

The authors wish to express appreciation to Dr. L. W. Sharp for aid in the revision of the manuscripts of this and the preceding paper. They are indebted to Dr. C. R. Burnham for furnishing unpublished data and for some of the material studied.



A New Method for the Study of Chromosome Rearrangements and Plotting of Chromosome Maps

T. S. PAINTER

Reprinted by author's and publisher's permission from *Science*, vol. 78, 1933, pp. 585-586.

A contribution to science can be made in many ways. I have pointed out examples of three different kinds of scientific paper in the previous pages of this collection. Painter's paper represents still another kind, for it is a report on the discovery of a new technique and the light thrown on problems of long standing as a consequence. The existence and activities of the enlarged, elongated, multiple, salivary gland chromosomes, and the facility with which they can be studied utilizing Painter's techniques, have led to increased knowledge of the structure of chromosomes and their behavior at meiosis.

A thorough review of the knowledge gained from the use of this technique is given by Painter in his paper, "Salivary Chromosomes and the Attack on the Gene," Journal of Heredity, vol. 25, 1934, pp. 464-476.

IT HAS LONG BEEN KNOWN THAT IN the functioning salivary glands of many dipteran larvae the chromosomes show an elongated and annulated structure. For the past year the writer has been studying such chromosomes, principally by the acetocarmine method, in larvae of *Drosophila melanogaster*. From this study the following conclusions are warranted:

(1) Each of the chromosomes has a definite and constant morphology and is made of segments, each of which has a characteristic pattern of chromatic lines or broader bands, which appear to run around the achromatic

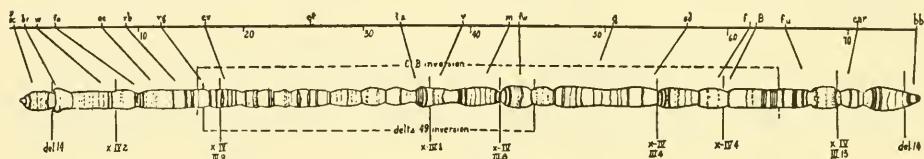
matrix. The same chromosomes, or characteristic parts thereof, may easily be recognized in different cells of an individual, or in different individuals of a species. If the position of one or more segments is shifted, by some form of dislocation (translocation, inversion, etc.), the exact morphological point (or points) of breakage can be determined and the segments identified in their new position. This discovery places in our hands, for the first time, a qualitative method of chromosome analysis and once the normal morphology of any given element is known, by studying chro-

mosome rearrangements of known genetic character, we can give morphological positions to gene loci and construct chromosome maps with far greater exactness than has been heretofore possible.

(2) In old larvae, homologous chromosomes undergo a process of somatic synapsis. This union is more than a simple apposition, for the elements pair up line for line in the most exact way and form one apparent structure. If one of the homologues carries an inverted section we get typical inversion figures, such as we would expect in meiosis. If one of the homologues is

deficient, at some point, the two mates unite except at the point of deficiency where the normal element usually buckles. Thus we can readily determine exactly how much of the one chromosome is missing. It is probable that the force which causes homologues to unite in salivary glands is the same that operates in meiosis, and while, so far as is known, these specialized chromosomes never divide, we can at least study how aberrant chromosomes unite at synapsis, a fact which should prove of great value to geneticists.

(3) In salivary glands the two arms



of the v-shaped autosomes appear as independent elements with no obvious connection between them. As a result, after somatic synapsis, we find six elements in the nucleus, not the haploid number.

(4) The inert region of the X-chromosome does not appear as an organic part of this element, nor does it show in any other as yet recognized form in the nucleus. Likewise, the only part of the Y-chromosome which has been identified is a short piece which, morphologically, is homologous to part of the right-hand end segment of the X. This part of the X (see figure) carries the normal allelomorph of bobbed. Either the inert material of both the X and Y has been eliminated during ontogeny, by diminution or some similar process, or this material exists in the salivary nuclei in some unrecognized form not visibly connected with the chromosomes. The inert area comprises about $\frac{3}{8}$ of the volume of the oögonial metaphase chromosomes.

The accompanying figure is a drawing of the X-chromosome made by uniting camera lucida sketches of various regions. Fine details are omitted. Above the figure, a crossover map having the same length as the X is shown. The symbols of gene loci, which have been located, are given together with lines showing their approximate morphological positions. The points of breakage are indicated on the X, with the name of the break given below. Thus, deletion 14 (at the left) broke the X between the loci of scute and broad. The morphological point of breakage is shown on the drawing, and, of course, scute must lie to the left of the break and broad to the right. In a similar way the position of other gene loci has been determined. Geneticists will be interested to note the morphological (and genetic) limits of C1B and delta 49 inversions as shown by the figure, and in the close correspondence between the cytological and crossover maps.

The writer has two articles in press, one dealing with the technique and the general morphological characters of the salivary chromosome, the second, a detailed study of the X-chromosome from which the drawing presented herewith was taken. Similar

studies of the autosomes have been under way for some time, and a number of students are at work on various cytological and genetic problems opened up by the new method of attack.



The Bar "Gene" a Duplication

CALVIN B. BRIDGES

Reprinted by publisher's permission from *Science*, vol. 83, 1936, pp. 210-211.

The facts of a field of science are similar to the pieces of a child's interlocking puzzle. All have their place, and it is usually if not always impossible to get them to go together out of sequence. On the other hand, when properly arranged, they fit very snugly indeed, and present a complete figure. This paper by Bridges combines the technique described by Painter (page 161) with the kind of investigation brilliantly carried out by Creighton and McClintock (page 155) and gives as a result a more detailed and informative explanation of the problem first investigated by Sturtevant (page 124). This is the way a science grows, bit by bit, and fact by fact.

THE NATURE OF THE BAR GENE HAS been the subject of extensive investigation and speculation since February, 1913, when Tice¹ found this reduced-eye mutant as a single male in the progeny of normal-eyed parents. The eye-reduction behaves as a sex-linked dominant, with a locus at 57.0, and has been one of the most important of all the sex-linked characters of *D. melanogaster*. A remarkable peculiarity

of the mutant is that occasionally the homozygous stock gives rise to a fly indistinguishable in appearance and genetic behavior from wild-type.² More rarely the stock gives rise to an even more extreme reduction in eye-size, a type which was called Ultra-Bar by Zeleny,³ who found it.

² May, H. G., *Biol. Bull.* 33:361-95, 1917.

³ Zeleny, C., *Jour. Exp. Zool.* 30:293-324, 1920.

¹ Tice, S. C., *Biol. Bull.* 26:221-51, 1914.

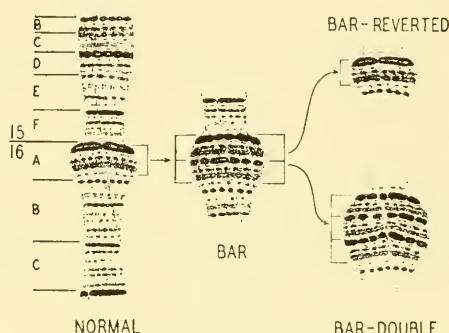
Sturtevant and Morgan⁴ and Sturtevant⁵ found that these two-way changes were the result of a novel type of "unequal" crossing-over, by which the two genes originally present in the two parental chromosomes both emerged in the same chromosome (Bar-double) while the other resultant chromosome was without Bar (Bar-reverted). The change from Bar to Bar-double was considered to be a single gene duplication, while the converse change, from Bar to Bar-reverted, corresponds to a one-gene deficiency. Since the Bar-reverted type proved to be indistinguishable from the normal unmutated wild-type, the gene present in Bar and lost in Bar-reverted must have itself corresponded to a new addition or one-gene duplication.⁶

Sturtevant⁵ found the unexpected relation that two Bar genes in the same chromosome (BB/B+) gave a greater reduction in the size of the Bar eye than did two Bar genes in opposite chromosomes (B/B), an intensification of action which he formulated as a "position effect." Dobzhansky⁷ interpreted his allelic Baroid mutant as a position effect due to the substitution of material at or near the Bar-locus (in the normal X) by material translocated from the right limb of chromosome 2, and the reduction in the Bar eye to the interaction between a gene in the X chromosome and the duplication.

A chance to clear up some of the puzzles as to the origin and behavior of Bar was offered by the salivary chromosomes. Study of the banding in

a stock of Bar (forked Bar) showed that an extra, short section of bands is present in excess of the normal complement, forming a duplication. The insertion point of this duplication is in the bulbous "turnip" segment, not far from the basal end of the X.⁸

The exact point of the insertion is ambiguous, for a reason which will appear below. The normal X in this region (see revised map below)



shows in sub-section 16A a heavy band, which in well-stretched chromosomes, or with certain fixations, is a clear doublet, usually with the halves united in a capsule, but occasionally completely separate. This is followed by a very faint dotted line, which can be seen only in the most favorable conditions. Next follows a fairly weak line which is distinctly "dotted" in texture, with the separate dots loosely connected across the width of the chromosome. Next follows closely a still fainter, diffuse, continuous-textured doublet, with the doubleness generally appearing as mere broadening. The last line of sub-section 16A is again a very faint dotted singlet. Sub-section 16B starts with a sharply discontinuous line of fairly heavy dots or vesicles and is a line very easy to recognize. The greatest width of the bulbous segment 16A is at the two

⁴ Sturtevant, A. H., and Morgan, T. H., *Science* 57:746-7, 1923.

⁵ Sturtevant, A. H., *Genetics* 10:117-47, 1925, (see p. 67 this vol.).

⁶ Wright, S., *Amer. Nat.* 63:479-80, 1929.

⁷ Dobzhansky, Th., *Genetics* 17:369-92, 1932.

⁸ Bridges, C. B., *Jour. Hered.* 26:60-4, 1935.

fairly weak bands, while a very sharp change in size occurs at the transition from 15F to 16A.

In the Bar chromosome the condition may be described observationally as the repetition of section 16A, with the exception of the final very faint dotted line. But the whole region of this bulb has undergone changes in the Bar chromosome as follows: the "puff" of the bulbous segment is more pronounced and its size is increased; the banding is more discontinuous by being broken into blocks and vesicles, and the regularity of synapsis is disturbed by oblique junctions. Thus, in Bar the heavy doublet following the last faint dotted line of 15F is more segmented than normal and more rarely shows its doubleness clearly. This tendency is more pronounced in the heavy broken line of the repeat seriation to the right. All the lines of the repeat seriation to the right differ from the corresponding lines of the initial seriation by being somewhat less intense, more broken, more diffuse and more confused in their synapsis relations.

In a forked non-Bar stock recently derived from the above forked Bar stock by breeding from the rare Bar-reversions, the banding was found to be precisely identical with that of unrelated normals as far as could be observed in excellent permanent preparations of well-stretched chromosomes.

In a forked Bar-double stock, similarly derived from the same f B stock by breeding from the very rare "Ultra-Bar" type of eye, it was found that the extra section observed in Bar was present still again, giving a thrice-repeated seriation in direct sequence. The changes differentiating Bar from normal were carried further in Bar-double, as follows: The size and puffiness of the bulbous regions was still greater, as well as the blockiness of the

banding and irregularity and obliqueness of the synapses. These disturbances were greatest in the middle one of the three seriations.

These findings enable the Bar "gene" to be reinterpreted as a section of inserted genes—a duplication. The production of Bar-double and of Bar-reverted is seen to be the insertion of this extra section twice, or conversely, its total loss—both presumably by a process of unequal crossing-over. That the section of bands should behave as a unit in this process is perhaps accounted for by the observation of oblique synapsis, especially frequent in Bar-double, where presumably one entire sequence synapses with another of a different position in the series of three. The oblique synapses were even more frequent in BB/B+, where one series in B+ has a choice of three series in BB, apparently usually synapsing with one or the other end series.

According to this interpretation the source of the duplication is the material directly adjacent to the repeat. But whether the point of insertion preceded the heavy doublet of 16A1 or the very faint final singlet of 16A5, can not be determined. If Bar is itself a repeat, a reason is thereby provided for its unique behavior of giving rise to Bar-double and Bar-reverted by oblique synapsis. Perhaps half of the Bar-reversions carry the original series and the other half the subsequent repeat restored to its original position.

On this interpretation, the "position effect"—the reinforcement of the action of one Bar gene by another in direct sequence next to it—has a visible cytological accompaniment in the increased size and puffiness, and the change in the character of the banding of both series in Bar as compared with normal and of all three series in Bar-double as compared to Bar itself. Part of this is presumably due to the

"rounding-up" tendency of the synaptic attraction along the chromosome in addition to the oblique attractions and the straight-across attractions.

The Bar-eye reduction is thus seen to be interpretable as the effect of increasing the action of certain genes by doubling or triplicating their number—a genic balance effect. But "position effects" are never excluded when duplications or other rearrangements are present, either in the wedging further apart of genes normally closer, or by the interaction with new neighbors. The respective shares attributable in the total effect to the genic-balance change and to the position-effect change seems to be at present a matter of taste.

Study of the Baroid translocation apparently shows that the break in X comes between the two halves of the heavy doublet of 16A1. The break in 2R follows directly after the heavy capsular doublet of 48C1. Thus a demonstrable basis is laid to Dobzhansky's interpretation of the Baroid eye-reduction as a position effect.⁷

The previously reported finding⁸ of the presence of "repeats" as a normal part of the chromosomes of *D. melanogaster*, and the suggestion that unequal crossing-over is probably the mechanism of production of some short repeats, thus have received ample verification by these direct observations on these processes in the case of Bar and its derivatives.



Genetic Control of Biochemical Reactions in *Neurospora*

G. W. BEADLE and E. L. TATUM

Reprinted by authors' and publisher's permission from *Proceedings of the National Academy of Sciences*, vol. 27, 1941, pp. 499–506.

In my remarks on Sewall Wright's paper (page 78) I pointed out that the approach implicit in that work was one of the most promising aspects of genetic research. The problems facing Wright and his students were enormous, however, and most of them stemmed from the difficulties of working with the mouse. It is practically impossible to tamper with the mouse's environment experimentally, and it is almost as bad to try to achieve uniformity of genotype in a large sample. The problems of the biochemistry and physiology of the gene required a new organism for successful investigation, although Wright's methods were still proving fruitful.

Beadle and Tatum found this new organism in *Neurospora*, and solved both of the difficulties. This mold is easily grown, reproduces rapidly asexually, which assures a plentiful supply of individuals of identical genotypes, and is excellent material for showing biochemical necessities, by varying the environmental supply. A later development in the technique has the virtue of demonstrating biochemical sequences in cellular metabolism. As a result, *Neurospora* has proven invaluable in both genetic and biochemical research, and another vast area of study has opened up.

FROM THE STANDPOINT OF PHYSIOLOGICAL genetics the development and functioning of an organism consist essentially of an integrated system of chemical reactions controlled in some manner by genes. It is entirely tenable to suppose that these genes which are themselves a part of the system, control or regulate specific reactions in the system either by acting directly as enzymes or by determining the specificities of enzymes.¹ Since the components of such a system are likely to be interrelated in complex ways, and since the synthesis of the parts of individual genes is presumably dependent on the functioning of other genes, it would appear that there must exist orders of directness of gene control ranging from simple one-to-one relations to relations of great complexity. In investigating the rôles of genes, the physiological geneticist usually attempts to determine the physiological and biochemical bases of already known hereditary traits. This approach, as made in the study of anthocyanin pigments in plants,² the

¹ The possibility that genes may act through the mediation of enzymes has been suggested by several authors. See Troland, L. T., *Amer. Nat.* 51:321-350, 1917; Wright, S., *Genetics* 12:530-569, 1927; and Haldane, J. B. S., in *Perspectives in Biochemistry*, Cambridge Univ. Press, pp. 1-10, 1937, for discussion and references.

² Onslow, Scott-Moncrieff and others, see review by Lawrence, W. J. C., and Price, J. R., *Biol. Rev.* 15:35-58, 1940.

fermentation of sugars by yeasts³ and a number of other instances,⁴ has established that many biochemical reactions are in fact controlled in specific ways by specific genes. Furthermore, investigations of this type tend to support the assumption that gene and enzyme specificities are of the same order.⁵ There are, however, a number of limitations inherent in this approach. Perhaps the most serious of these is that the investigator must in general confine himself to a study of non-lethal heritable characters. Such characters are likely to involve more or less non-essential so-called "terminal" reactions.⁵ The selection of these for genetic study was perhaps responsible for the now rapidly disappearing belief that genes are concerned only with the control of "superficial" characters. A second difficulty, not unrelated to the first, is that the standard approach to the problem implies the use of the characters with visible manifestations. Many such characters involve morphological variations, and these are likely

³ Winge, O. and Laustsen, O., *Compt. rend. Lab. Carlsberg, Serie physiol.* 22:337-352, 1939.

⁴ See Goldschmidt, R., *Physiological Genetics*, McGraw-Hill, pp. 1-375, 1939; and Beadle, G. W. and Tatum, E. L., *Amer. Nat.* 75:107-116, 1941, for discussion and references.

⁵ See Sturtevant, A. H. and Beadle, G. W., *An Introduction to Genetics*, Saunders, pp. 1-391, 1931; and Beadle, G. W. and Tatum, E. L., *loc. cit.*, footnote 4.

to be based on systems of biochemical reactions so complex as to make analysis exceedingly difficult.

Considerations such as those just outlined have led us to investigate the general problem of the genetic control of developmental and metabolic reactions by reversing the ordinary procedure and, instead of attempting to work out the chemical bases of known genetic characters, to set out to determine if and how genes control known biochemical reactions. The ascomycete *Neurospora* offers many advantages for such an approach and is well suited to genetic studies.⁶ Accordingly, our program has been built around this organism. The procedure is based on the assumption that x-ray treatment will induce mutations in genes concerned with the control of known specific chemical reactions. If the organism must be able to carry out a certain chemical reaction to survive on a given medium, a mutant unable to do this will obviously be lethal on this medium. Such a mutant can be maintained and studied, however, if it will grow on a medium to which has been added the essential product of the genetically blocked reaction. The experimental procedure based on this reasoning can best be illustrated by considering a hypothetical example. Normal strains of *Neurospora crassa* are able to use sucrose as a carbon source, and are therefore able to carry out the specific and enzymatically controlled reaction involved in the hydrolysis of this sugar. Assuming this reaction to be genetically controlled, it should be possible to induce a gene to mutate to a condition such that the organism could no longer carry out sucrose hydrolysis. A strain carrying

this mutant would then be unable to grow on a medium containing sucrose as a sole carbon source but should be able to grow on a medium containing some other normally utilizable carbon source. In other words, it should be possible to establish and maintain such a mutant strain on a medium containing glucose and detect its inability to utilize sucrose by transferring it to a sucrose medium.

Essentially similar procedures can be developed for a great many metabolic processes. For example, ability to synthesize growth factors (vitamins), amino acids and other essential substances should be lost through gene mutation if our assumptions are correct. Theoretically, any such metabolic deficiency can be "by-passed" if the substance lacking can be supplied in the medium and can pass cell walls and protoplasmic membranes.

In terms of specific experimental practice, we have devised a procedure in which x-rayed single-spore cultures are established on a so-called "complete" medium, i.e., one containing as many of the normally synthesized constituents of the organism as is practicable. Subsequently these are tested by transferring them to a "minimal" medium, i.e., one requiring the organism to carry on all the essential syntheses of which it is capable. In practice the complete medium is made up of agar, inorganic salts, malt extract, yeast extract and glucose. The minimal medium contains agar (optional), inorganic salts and biotin, and a disaccharide, fat or more complex carbon source. Biotin, the one growth factor that wild-type *Neurospora* strains cannot synthesize,⁷ is supplied in the form

⁶ Dodge, B. O., *Jour. Agric. Res.* 35:289-305, 1927; and Lindegren, C. C., *Bull. Torrey Bot. Club* 59:85-102, 1932.

⁷ In so far as we have carried them, our investigations on the vitamin requirements of *Neurospora* corroborate those of Butler, E. T., Robbins, W. J., and Dodge, B. O., *Science* 94:262-263, 1941.

of a commercial concentrate containing 100 micrograms of biotin per cc.⁸ Any loss of ability to synthesize an essential substance present in the complete medium and absent in the minimal medium is indicated by a strain growing on the first and failing to grow on the second medium. Such strains are then tested in a systematic manner to determine what substance or substances they are unable to synthesize. These subsequent tests include attempts to grow mutant strains on the minimal medium with (1) known vitamins added, (2) amino acids added or (3) glucose substituted for the more complex carbon source of the minimal medium.

Single ascospore strains are individually derived from perithecia of *N. crassa* and *N. sitophila* x-rayed prior to meiosis. Among approximately 2000 such strains, three mutants have been found that grow essentially normally on the complete medium and scarcely at all on the minimal medium with sucrose as the carbon source. One of these strains (*N. sitophila*) proved to be unable to synthesize vitamin B₆ (pyridoxine). A second strain (*N. sitophila*) turned out to be unable to synthesize vitamin B₁ (thiamine). Additional tests show that this strain is able to synthesize the pyrimidine half of the B₁ molecule but not the thiazole half. If thiazole alone is added to the minimal medium, the strain grows essentially normally. A third strain (*N. crassa*) has been found to be unable to synthesize para-aminobenzoic acid. This mutant strain appears to be entirely normal when grown on the minimal medium to which *p*-aminobenzoic acid has been added. Only in the case of the "pyridoxinless" strain has an analysis of the inheritance of

the induced metabolic defect been investigated. For this reason detailed accounts of the thiamine-deficient and *p*-aminobenzoic acid-deficient strains will be deferred.

Qualitative studies indicate clearly that the pyridoxinless mutant, grown on a medium containing one microgram or more of synthetic vitamin B₆ hydrochloride per 25 cc. of medium, closely approaches in rate and characteristics of growth normal strains grown on a similar medium with no B₆. Lower concentrations of B₆ give intermediate growth rates. A preliminary investigation of the quantitative dependence of growth of the mutant on vitamin B₆ in the medium gave the results summarized in table 1. Additional experiments have given results essentially similar but in only approxi-

TABLE 1

Growth of Pyridoxinless Strain of *N. sitophila* on Liquid Medium Containing Inorganic Salts,⁹ 1% Sucrose, and 0.004 Microgram Biotin per Cc. Temperature 25°C. Growth Period, 6 Days from Inoculation with Conidia

Micrograms B ₆ per 25 cc. medium	Strain	Dry weight mycelia, mg.
0	Normal	76.7
0	Pyridoxinless	1.0
0.01	"	4.2
0.03	"	5.7
0.1	"	13.7
0.3	"	25.5
1.0	"	81.1
3.0	"	81.1
10.0	"	65.4
30.0	"	82.4

⁹ Throughout our work with *Neurospora*, we have used as a salt mixture the one designated number 3 by Fries, N., *Symbolae Bot. Upsalienses*, vol. 3, No. 2, 1-188, 1938. This has the following composition: NH₄ tartrate, 5 g.; NH₄NO₃, 1 g.; KH₂PO₄, 1 g.; MgSO₄ · 7H₂O, 0.5 g.; NaCl, 0.1 g.; CaCl₂, 0.1 g.; FeCl₃, 10 drops 1% solution; H₂O, 1 l. The tartrate cannot be used as a carbon source by *Neurospora*.

⁸ The biotin concentrate used was obtained from the S. M. A. Corporation, Chagrin Falls, Ohio.

mate quantitative agreement with those of table 1. It is clear that additional study of the details of culture conditions is necessary before rate of weight increase of this mutant can be used as an accurate assay for vitamin B₆.

It has been found that the progression of the frontier of mycelia of *Neurospora* along a horizontal glass culture tube half filled with an agar medium provides a convenient method of investigating the quantitative effects of

growth factors. Tubes of about 13 mm. inside diameter and about 40 cm. in length are used. Segments of about 5 cm. at the two ends are turned up at an angle of about 45°. Agar medium is poured in so as to fill the tube about half full and is allowed to set with the main segment of the tube in a horizontal position. The turned up ends of the tube are stoppered with cotton plugs. Inoculations are made at one end of the agar surface and the position of the advancing front recorded at con-

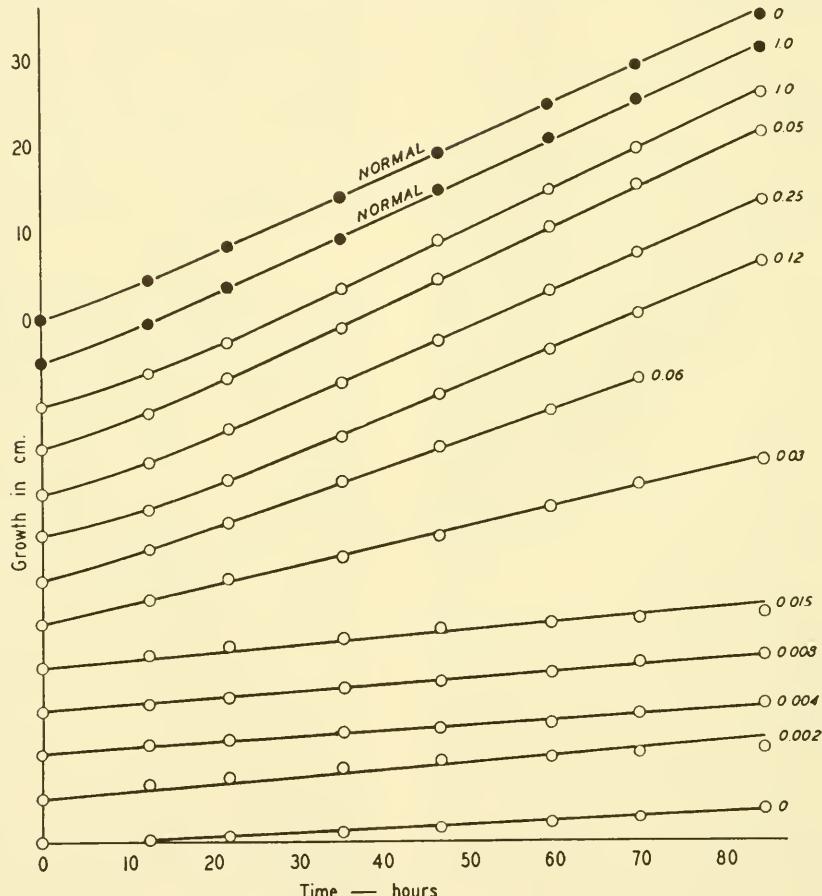


Fig. 1. Growth of normal (top two curves) and pyridoxinless (remaining curves) strains of *Neurospora sitophila* in horizontal tubes. The scale on the ordinate is shifted a fixed amount for each successive curve in the series. The figures at the right of each curve indicate concentration of pyridoxine (B₆) in micrograms per 25 cc. medium.

venient intervals. The frontier formed by the advancing mycelia is remarkably well defined, and there is no difficulty in determining its position to within a millimeter or less. Progression along such tubes is strictly linear with time and the rate is independent of tube length (up to 1.5 meters). The rate is not changed by reducing the inside tube diameter to 9 mm., or by sealing one or both ends. It therefore appears that gas diffusion is in no way limiting in such tubes.

The results of growing the pyridoxinless strain in horizontal tubes in which the agar medium contained varying amounts of B_6 are shown graphically in figures 1 and 2. Rate of

progression is clearly a function of vitamin B_6 concentration in the medium.¹⁰ It is likewise evident that there is no significant difference in rate between the mutant supplied with B_6 and the normal strain growing on a medium without this vitamin. These results are consistent with the assumption that the primary physiological difference between pyridoxinless and normal strains is the inability of the former to carry out the synthesis of vitamin B_6 . There is certainly more than one step in this synthesis and accordingly the gene differential involved is presumably concerned with only one specific step in the biosynthesis of vitamin B_6 .

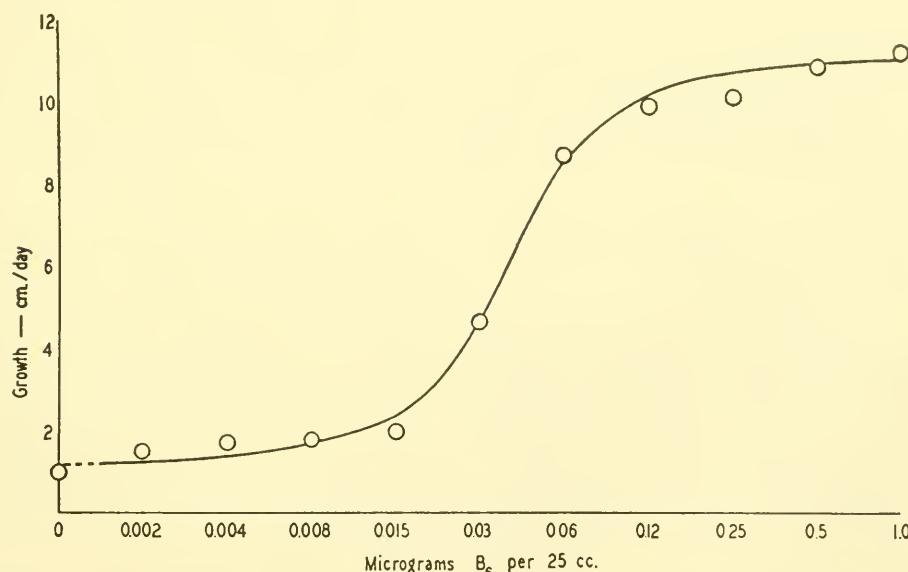


Fig. 2. The relation between growth rate (cm./day) and vitamin B_6 concentration.

In order to ascertain the inheritance of the pyridoxinless character, crosses between normal and mutant strains were made. The techniques for hybridization and ascospore isolation have been worked out and described by Dodge, and by Lindegren.⁶ The ascospores from 24 asci of the cross

were isolated and their positions in the asci recorded. For some unknown rea-

¹⁰ It is planned to investigate further the possibility of using the growth of *Neurospora* strains in the described tube as a basis of vitamin assay, but it should be emphasized that such additional investigation is essential in order to determine the reproducibility and reliability of the method.

son, most of these failed to germinate. From seven asci, however, one or more spores germinated. These were grown on a medium containing glucose, malt extract and yeast extract, and in this they all grew normally. The normal and mutant cultures were differen-

tiated by growing them on a B₆ deficient medium. On this medium the mutant cultures grew very little, while the non-mutant ones grew normally. The results are summarized in table 2. It is clear from these rather limited data that this inability to synthesize

TABLE 2

Results of Classifying Single Ascospore Cultures from the Cross of
Pyridoxinless and Normal *N. sitophila*

Ascus Number	1	2	3	4	5	6	7	8
17	—	pdx	pdx	pdx	N	N	N	—
18	—	—	N	N	—	—	pdx	pdx
19	—	pdx	—	—	—	—	—	N
20	—	—	N	—	—	—	—	pdx
22	—	—	N	—	—	—	—	—
23	—	*	*	*	N	N	pdx	pdx
24	N	N	N	N	pdx	pdx	pdx	pdx

N, normal growth on B₆-free medium. pdx, slight growth on B₆-free medium. Failure of ascospore germination indicated by dash.

* Spores 2, 3 and 4 isolated but positions confused. Of these, two germinated and both proved to be mutants.

vitamin B₆ is transmitted as it should be if it were differentiated from normal by a single gene.

The preliminary results summarized above appear to us to indicate that the approach outlined may offer considerable promise as a method of learning more about how genes regulate development and function. For example, it should be possible, by finding a number of mutants unable to carry out a particular step in a given synthesis, to determine whether only one gene is ordinarily concerned with the immediate regulation of a given specific chemical reaction.

It is evident, from the standpoints of biochemistry and physiology, that the method outlined is of value as a technique for discovering additional substances of physiological significance. Since the complete medium used can be made up with yeast ex-

tract or with an extract of normal *Neurospora*, it is evident that if, through mutation, there is lost the ability to synthesize an essential substance, a test strain is thereby made available for use in isolating the substance. It may, of course, be a substance not previously known to be essential for the growth of any organism. Thus we may expect to discover new vitamins, and in the same way, it should be possible to discover additional essential amino acids if such exist. We have, in fact, found a mutant strain that is able to grow on a medium containing Difco yeast extract but unable to grow on any of the synthetic media we have so far tested. Evidently some growth factor present in yeast and as yet unknown to us is essential for *Neurospora*.

Summary—A procedure is outlined by which, using *Neurospora*, one can

discover and maintain x-ray induced mutant strains which are characterized by their inability to carry out specific biochemical processes.

Following this method, three mutant strains have been established. In one of these the ability to synthesize vitamin B₆ has been wholly or largely lost. In a second the ability to synthesize the thiazole half of the vitamin B₁ molecule is absent, and in the third para-aminobenzoic acid is not synthesized. It is therefore clear that all of these substances are essential growth factors for *Neurospora*.¹¹

Growth of the pyridoxinless mutant (a mutant unable to synthesize vitamin B₆) is a function of the B₆ content of the medium on which it is grown. A method is described for measuring the growth by following linear progression of the mycelia along a horizontal tube half filled with an agar medium.

Inability to synthesize vitamin B₆ is

apparently differentiated by a single gene from the ability of the organism to elaborate this essential growth substance.

Note: Since the manuscript of this paper was sent to press it has been established that inability to synthesize both thiazole and aminobenzoic acid is also inherited as though differentiated from normal by single genes.

Work supported in part by a grant from the Rockefeller Foundation. The authors are indebted to Doctors B. O. Dodge, C. C. Lindegren and W. S. Malloch for stocks and for advice on techniques, and to Miss Caryl Parker for technical assistance.

¹¹ The inference that the three vitamins mentioned are essential for the growth of normal strains is supported by the fact that an extract of the normal strain will serve as a source of vitamin for each of the mutant strains.



Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types

Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from *Pneumococcus* Type III

OSWALD T. AVERY, COLIN M. MACLEOD,
and MACLYN MCCARTY

Reprinted by authors' and publisher's permission from *Journal of Experimental Medicine*, vol. 79, 1944, pp. 137-158.

This paper is indicative of one of the current trends in genetics, which has developed many divergent pathways. The reader who has

followed the sequence of papers in this volume will find himself plunged into an entirely new vocabulary and terminology by this work. The genetic facts and phenomena he has become familiar with in the other papers are all here, but their setting is unfamiliar. It is like encountering a traffic policeman in a foreign country. His duties are similar to those at home, but the details of his performance are novel, and slightly confusing. One must understand the local situation before he drives successfully, and so it is here. The effort necessary to follow the intricacies of bacteriological detail is rewarded by a new insight into genetic mechanisms and structure. This will be found equally true of the paper by Benzer on p. 271.

This paper is of outstanding importance to genetics, of course, for the precise way in which it shows DNA to be a primary hereditary material. For the first time a definable chemical compound plays a role in changing the hereditary makeup of an organism, and, once it has played this role, shows also that it is capable of reproducing itself precisely, "in amounts far in excess of that originally added" (p. 190). The experimental data and evidence the authors have marshalled to prove this point are quite impressive, and the reader should note the multitude of techniques and apparatus involved. Genetic investigations on micro-organisms are exceedingly complex, but are also exceedingly rewarding.

BIOLOGISTS HAVE LONG ATTEMPTED BY chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microorganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. This phenomenon was first described by Griffith (1) who succeeded in transforming an attenuated and non-encapsulated (R) variant derived from one specific type into fully encapsulated and virulent (S) cells of a heterologous specific type. A typical instance will suffice to illustrate the techniques originally used and serve to indicate the wide variety of transformations that are possible within the limits of this bacterial species.

Griffith found that mice injected subcutaneously with a small amount of a living R culture derived from *Pneumococcus Type II* together with a large inoculum of heat-killed Type III (S) cells frequently succumbed to infection, and that the heart's blood of these animals yielded Type III pneumococci in pure culture. The fact that the R strain was avirulent and incapable by itself of causing fatal bacteremia and the additional fact that the heated suspension of Type III cells contained no viable organisms brought convincing evidence that the R forms growing under these conditions had newly acquired the capsular structure and biological specificity of Type III pneumococci.

The original observations of Griffith were later confirmed by Neufeld and Levinthal (2), and by Baurhenn (3) abroad, and by Dawson (4) in this laboratory. Subsequently Dawson and Sia (5) succeeded in inducing transfor-

mation *in vitro*. This they accomplished by growing R cells in a fluid medium containing anti-R serum and heat-killed encapsulated S cells. They showed that in the test tube as in the animal body transformation can be selectively induced, depending on the type specificity of the S cells used in the reaction system. Later, Alloway (6) was able to cause specific transformation *in vitro* using sterile extracts of S cells from which all formed elements and cellular debris had been removed by Berkefeld filtration. He thus showed that crude extracts containing active transforming material in soluble form are as effective in inducing specific transformation as are the intact cells from which the extracts were prepared.

Another example of transformation which is analogous to the interconvertibility of pneumococcal types lies in the field of viruses. Berry and Debrick (7) succeeded in changing the virus of rabbit fibroma (Shope) into that of infectious myxoma (Sanarelli). These investigators inoculated rabbits with a mixture of active fibroma virus together with a suspension of heat-inactivated myxoma virus and produced in the animals the symptoms and pathological lesions characteristic of infectious myxomatosis. On subsequent animal passage the transformed virus was transmissible and induced myxomatous infection typical of the naturally occurring disease. Later Berry (8) was successful in inducing the same transformation using a heat-inactivated suspension of washed elementary bodies of myxoma virus. In the case of these viruses the methods employed were similar in principle to those used by Griffith in the transformation of pneumococcal types. These observations have subsequently been confirmed by other investigators (9).

The present paper is concerned with

a more detailed analysis of the phenomenon of transformation of specific types of Pneumococcus. The major interest has centered in attempts to isolate the active principle from crude bacterial extracts and to identify if possible its chemical nature or at least to characterize it sufficiently to place it in a general group of known chemical substances. For purposes of study, the typical example of transformation chosen as a working model was the one with which we have had most experience and which consequently seemed best suited for analysis. This particular example represents the transformation of a non-encapsulated R variant of Pneumococcus Type II to Pneumococcus Type III.

EXPERIMENTAL

Transformation of pneumococcal types *in vitro* requires that certain cultural conditions be fulfilled before it is possible to demonstrate the reaction even in the presence of a potent extract. Not only must the broth medium be optimal for growth but it must be supplemented by the addition of serum or serous fluid known to possess certain special properties. Moreover, the R variant, as will be shown later, must be in the reactive phase in which it has the capacity to respond to the transforming stimulus. For purposes of convenience these several components as combined in the transforming test will be referred to as the *reaction system*. Each constituent of this system presented problems which required clarification before it was possible to obtain consistent and reproducible results. The various components of the system will be described in the following order: (1) nutrient broth, (2) serum or serous fluid, (3) strain of R Pneumococcus, and (4) extraction, purification, and chemical nature of the transforming principle.

1. *Nutrient Broth*.—Beef heart infusion broth containing 1 per cent neopeptone with no added dextrose and adjusted to an initial pH of 7.6-7.8 is used as the basic medium. Individual lots of broth show marked and unpredictable variations in the property of supporting transformation. It has been found, however, that charcoal adsorption, according to the method described by MacLeod and Mirick (10) for removal of sulfonamide inhibitors, eliminates to a large extent these variations; consequently this procedure is used as routine in the preparation of consistently effective broth for titrating the transforming activity of extracts.

2. *Serum or Serous Fluid*.—In the first successful experiments on the induction of transformation *in vitro*, Dawson and Sia (5) found that it was essential to add serum to the medium. Anti-R pneumococcal rabbit serum was used because of the observation that reversion of an R pneumococcus to the homologous S form can be induced by growth in a medium containing anti-R serum. Alloway (6) later found that ascitic or chest fluid and normal swine serum, all of which contain R antibodies, are capable of replacing antipneumococcal rabbit serum in the reaction system. Some form of serum is essential, and to our knowledge transformation *in vitro* has never been effected in the absence of serum or serous fluid.

In the present study human pleural or ascitic fluid has been used almost exclusively. It became apparent, however, that the effectiveness of different lots of serum varied and that the differences observed were not necessarily dependent upon the content of R antibodies, since many sera of high titer were found to be incapable of supporting transformation. This fact sug-

gested that factors other than R antibodies are involved.

It has been found that sera from various animal species, irrespective of their immune properties, contain an enzyme capable of destroying the transforming principle in potent extracts. The nature of this enzyme and the specific substrate on which it acts will be referred to later in this paper. This enzyme is inactivated by heating the serum at 60°-65°C., and sera heated at temperatures known to destroy the enzyme are often rendered effective in the transforming system. Further analysis has shown that certain sera in which R antibodies are present and in which the enzyme has been inactivated may nevertheless fail to support transformation. This fact suggests that still another factor in the serum is essential. The content of this factor varies in different sera, and at present its identity is unknown.

There are at present no criteria which can be used as a guide in the selection of suitable sera or serous fluids except that of actually testing their capacity to support transformation. Fortunately, the requisite properties are stable and remain unimpaired over long periods of time; and sera that have been stored in the refrigerator for many months have been found on retesting to have lost little or none of their original effectiveness in supporting transformation.

The recognition of these various factors in serum and their rôle in the reaction system has greatly facilitated the standardization of the cultural conditions required for obtaining consistent and reproducible results.

3. *The R Strain (R36A)*.—The unencapsulated R strain used in the present study was derived from a virulent "S" culture of Pneumococcus Type II. It will be recalled that irrespective

of type derivation all "R" variants of *Pneumococcus* are characterized by the lack of capsule formation and the consequent loss of both type specificity and the capacity to produce infection in the animal body. The designation of these variants as R forms has been used to refer merely to the fact that on artificial media the colony surface is "rough" in contrast to the smooth, glistening surface of colonies of encapsulated S cells.

The R strain referred to above as R36A was derived by growing the parent S culture of *Pneumococcus* Type II in broth containing Type II antipneumococcus rabbit serum for 36 serial passages and isolating the variant thus induced. The strain R36A has lost all the specific and distinguishing characteristics of the parent S organisms and consists only of attenuated and non-encapsulated R variants. The change S → R is often a reversible one provided the R cells are not too far "degraded." The reversion of the R form to its original specific type can frequently be accomplished by successive animal passages or by repeated serial subculture in anti-R serum. When reversion occurs under these conditions, however, the R culture invariably reverts to the encapsulated form of the same specific type as that from which it was derived (11). Strain R36A has become relatively fixed in the R phase and has never spontaneously reverted to the Type II S form. Moreover, repeated attempts to cause it to revert under the conditions just mentioned have in all instances been unsuccessful.

The reversible conversion of S ⇌ R within the limits of a single type is quite different from the transformation of one specific type of *Pneumococcus* into another specific type through the R form. Transformation of types has never been observed to occur spontaneously and has been induced experimentally only by the spe-

cial techniques outlined earlier in this paper. Under these conditions, the enzymatic synthesis of a chemically and immunologically different capsular polysaccharide is specifically oriented and selectively determined by the specific type of S cells used as source of the transforming agent.

In the course of the present study it was noted that the stock culture of R36 on serial transfers in blood broth undergoes spontaneous dissociation giving rise to a number of other R variants which can be distinguished one from another by colony form. The significance of this in the present instance lies in the fact that of four different variants isolated from the parent R culture only one (R36A) is susceptible to the transforming action of potent extracts, while the others fail to respond and are wholly inactive in this regard. The fact that differences exist in the responsiveness of different R variants to the same specific stimulus emphasizes the care that must be exercised in the selection of a suitable R variant for use in experiments on transformation. The capacity of this R strain (R36A) to respond to a variety of different transforming agents is shown by the readiness with which it can be transformed to Types I, III, VI, or XIV, as well as to its original type (Type II), to which, as pointed out, it has never spontaneously reverted.

Although the significance of the following fact will become apparent later on, it must be mentioned here that pneumococcal cells possess an enzyme capable of destroying the activity of the transforming principle. Indeed, this enzyme has been found to be present and highly active in the autolysates of a number of different strains. The fact that this intracellular enzyme is released during autolysis may explain, in part at least, the observation of Dawson and Sia (5) that it is essential in bringing about transformation in the test tube to use a small inoculum of young and actively growing R cells. The irregularity of the results and often the failure to induce transfor-

mation when large inocula are used may be attributable to the release from autolyzing cells of an amount of this enzyme sufficient to destroy the transforming principle in the reaction system.

In order to obtain consistent and reproducible results, two facts must be borne in mind: first, that an R culture can undergo spontaneous dissociation and give rise to other variants which have lost the capacity to respond to the transforming stimulus; and secondly, that pneumococcal cells contain an intracellular enzyme which when released destroys the activity of the transforming principle. Consequently, it is important to select a responsive strain and to prevent as far as possible the destructive changes associated with autolysis.

Method of Titration of Transforming Activity.—In the isolation and purification of the active principle from crude extracts of pneumococcal cells it is desirable to have a method for determining quantitatively the transforming activity of various fractions.

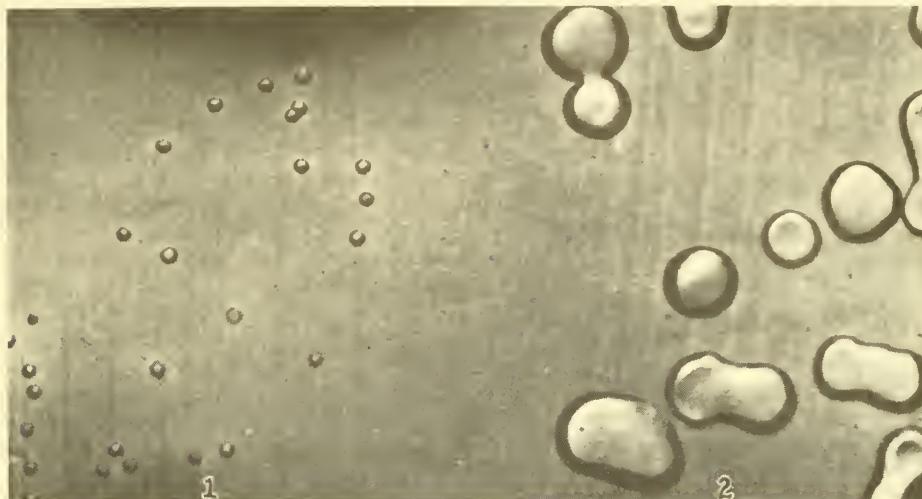
The experimental procedure used is as follows: Sterilization of the material to be tested for activity is accomplished by the use of alcohol since it has been found that this reagent has no effect on activity. A measured volume of extract is precipitated in a sterile centrifuge tube by the addition of 4 to 5 volumes of absolute ethyl alcohol, and the mixture is allowed to stand 8 or more hours in the refrigerator in order to effect sterilization. The alcohol precipitated material is centrifuged, the supernatant discarded, and the tube containing the precipitate is allowed to drain for a few minutes in the inverted position to remove excess alcohol. The mouth of the tube is then carefully flamed and a dry, sterile cotton plug is inserted. The precipitate is redissolved in the original volume of saline. Sterilization of active material by this technique has invariably proved effective. This pro-

cure avoids the loss of active substance which may occur when the solution is passed through a Berkefeld filter or is heated at the high temperatures required for sterilization.

To the charcoal-adsorbed broth described above is added 10 per cent of the sterile ascitic or pleural fluid which has previously been heated at 60°C. for 30 minutes, in order to destroy the enzyme known to inactivate the transforming principle. The enriched medium is distributed under aseptic conditions in 2.0 cc. amounts in sterile tubes measuring 15 × 100 mm. The sterilized extract is diluted serially in saline neutralized to pH 7.2–7.6 by addition of 0.1 N NaOH, or it may be similarly diluted in M/40 phosphate buffer, pH 7.4. 0.2 cc. of each dilution is added to at least 3 or 4 tubes of the serum medium. The tubes are then seeded with a 5 to 8 hour blood broth culture of R36A. 0.05 cc. of a 10⁻⁴ dilution of this culture is added to each tube, and the cultures are incubated at 37°C. for 18 to 24 hours.

The anti-R properties of the serum in the medium cause the R cells to agglutinate during growth, and clumps of the agglutinated cells settle to the bottom of the tube leaving a clear supernatant. When transformation occurs, the encapsulated S cells, not being affected by these antibodies, grow diffusely throughout the medium. On the other hand, in the absence of transformation the supernatant remains clear, and only sedimented growth of R organisms occurs. This difference in the character of growth makes it possible by inspection alone to distinguish tentatively between positive and negative results. As routine all the cultures are plated on blood agar for confirmation and further bacteriological identification. Since the extracts used in the present study were derived from *Pneumococcus Type III*, the differentiation between the colonies of the original R organism and those of the transformed S cells is especially striking, the latter

being large, glistening, mucoid colonies typical of *Pneumococcus* Type III. Figs. 1 and 2 illustrate these differences in colony form.



Figs. 1 and 2. (1) Colonies of the R variant (R36A) derived from *Pneumococcus* type II. Plated on blood agar from a culture grown in serum broth in the absence of the transforming substance. X3.5. (2) Colonies on blood agar of the same cells after induction of transformation during growth in the same medium with the addition of active glistening, mucoid colonies shown are characteristic of *Pneumococcus* Type III and readily distinguishable from the small, rough colonies of the parent R strain illustrated in Fig. 1. X3.5. (The photograph was made by Mr. Joseph B. Haulenbeek.)

Preparative Methods

Source Material.—In the present investigation a stock laboratory strain of *Pneumococcus* Type III (A66) has been used as source material for obtaining the active principle. Mass cultures of these organisms are grown in 50 to 75 liter lots of plain beef heart infusion broth. After 16 to 18 hours' incubation at 37°C. the bacterial cells are collected in a steam-driven sterilizable Sharples centrifuge. The centrifuge is equipped with cooling coils immersed in ice water so that the culture fluid is thoroughly chilled before flowing into the machine. This procedure retards autolysis during the course of centrifugation. The sedimented bacteria are removed from the collecting cylinder and resuspended in approximately 150 cc. of chilled saline (0.85 per cent NaCl), and care is taken that all clumps are thor-

oughly emulsified. The glass vessel containing the thick, creamy suspension of cells is immersed in a water bath, and the temperature of the suspension rapidly raised to 65°C. During the heating process the material is constantly stirred, and the temperature maintained at 65°C. for 30 minutes. Heating at this temperature inactivates the intracellular enzyme known to destroy the transforming principle.

Extraction of Heat-Killed Cells.—Although various procedures have been used, only that which has been found most satisfactory will be described here. The heat-killed cells are washed with saline 3 times. The chief value of the washing process is to remove a large excess of capsular polysaccharide together with much of the protein, ribonucleic acid, and somatic "C" polysaccharide. Quantitative titrations of transforming

activity have shown that not more than 10 to 15 per cent of the active material is lost in the washing, a loss which is small in comparison to the amount of inert substances which are removed by this procedure.

After the final washing, the cells are extracted in 150 cc. of saline containing sodium desoxycholate in final concentration of 0.5 per cent by shaking the mixture mechanically 30 to 60 minutes. The cells are separated by centrifugation, and the extraction process is repeated 2 or 3 times. The desoxycholate extracts prepared in this manner are clear and colorless. These extracts are combined and precipitated by the addition of 3 to 4 volumes of absolute ethyl alcohol. The sodium desoxycholate being soluble in alcohol remains in the supernatant and is thus removed at this step. The precipitate forms a fibrous mass which floats to the surface of the alcohol and can be removed directly by lifting it out with a spatula. The excess alcohol is drained from the precipitate which is then redissolved in about 50 cc. of saline. The solution obtained is usually viscous, opalescent, and somewhat cloudy.

Deproteinization and Removal of Capsular Polysaccharide.—The solution is then deproteinized by the chloroform method described by Sevag (12). The procedure is repeated 2 or 3 times until the solution becomes clear. After this preliminary treatment the material is reprecipitated in 3 to 4 volumes of alcohol. The precipitate obtained is dissolved in a larger volume of saline (150 cc.) to which is added 3 to 5 mg. of a purified preparation of the bacterial enzyme capable of hydrolyzing the Type III capsular polysaccharide (13). The mixture is incubated at 37°C., and the destruction of the capsular polysaccharide is determined by serological tests with Type III antibody solution prepared by dissociation of immune precipitate according to the method described by Liu and Wu (14). The advantages of using the antibody solution for this purpose are that it does not react with other serologically active substances in the extract and that it selectively detects the presence of the capsular

polysaccharide in dilutions as high as 1:6,000,000. The enzymatic breakdown of the polysaccharide is usually complete within 4 to 6 hours, as evidenced by the loss of serological reactivity. The digest is then precipitated in 3 to 4 volumes of ethyl alcohol, and the precipitate is redissolved in 50 cc. of saline. Deproteinization by the chloroform process is again used to remove the added enzyme protein and remaining traces of pneumococcal protein. The procedure is repeated until no further film of protein-chloroform gel is visible at the interface.

Alcohol Fractionation.—Following deproteinization and enzymatic digestion of the capsular polysaccharide, the material is repeatedly fractionated in ethyl alcohol as follows. Absolute ethyl alcohol is added dropwise to the solution with constant stirring. At a critical concentration varying from 0.8 to 1.0 volume of alcohol the active material separates out in the form of fibrous strands that wind themselves around the stirring rod. This precipitate is removed on the rod and washed in a 50 per cent mixture of alcohol and saline. Although the bulk of active material is removed by fractionation at the critical concentration, a small but appreciable amount remains in solution. However, upon increasing the concentration of alcohol to 3 volumes, the residual fraction is thrown down together with inert material in the form of a flocculent precipitate. This flocculent precipitate is taken up in a small volume of saline (5 to 10 cc.) and the solution again fractionated by the addition of 0.8 to 1.0 volume of alcohol. Additional fibrous material is obtained which is combined with that recovered from the original solution. Alcoholic fractionation is repeated 4 to 5 times. The yield of fibrous material obtained by this method varies from 10 to 25 mg. per 75 liters of culture and represents the major portion of active material present in the original crude extract.

Effect of Temperature.—As a routine procedure all steps in purification were carried out at room temperature unless specifically stated otherwise. Because of the theoretical advantage of working at

low temperature in the preparation of biologically active material, the purification of one lot (preparation 44) was carried out in the cold. In this instance all the above procedures with the exception of desoxycholate extraction and enzyme treatment were conducted in a cold room maintained at 0-4°C. This preparation proved to have significantly higher activity than did material similarly prepared at room temperature.

Desoxycholate extraction of the heat-killed cells at low temperature is less efficient and yields smaller amounts of the active fraction. It has been demonstrated that higher temperatures facilitate extraction of the active principle, although activity is best preserved at low temperatures.

Analysis of Purified Transforming Material

General Properties.—Saline solutions containing 0.5 to 1.0 mg. per cc. of the purified substance are colorless and clear in diffuse light. However, in strong transmitted light the solution is not entirely clear and when stirred exhibits a silky sheen. Solutions at these concentrations are highly viscous.

Purified material dissolved in physiological salt solution and stored at 2-4°C. retains its activity in undiminished titer for at least 3 months. However, when dissolved in distilled water, it rapidly decreases in activity and becomes completely inert within a few days. Saline solutions stored in the frozen state in a CO₂ ice box (-70°C.) retain full potency for several months. Similarly, material precipitated from saline solution by alcohol and stored under the supernatant remains active over a long period of time. Partially purified material can be preserved by drying from the frozen state in the lyophile apparatus. However, when the same procedure is used for the preservation of the highly purified substance, it is found that the material undergoes changes resulting in de-

crease in solubility and loss of activity.

The activity of the transforming principle in crude extracts withstands heating for 30 to 60 minutes at 65°C. Highly purified preparations of active material are less stable, and some loss of activity occurs at this temperature. A quantitative study of the effect of heating purified material at higher temperatures has not as yet been made. Alloway (6), using crude extracts prepared from Type III pneumococcal cells, found that occasionally activity could still be demonstrated after 10 minutes' exposure in the water bath to temperatures as high as 90°C.

The procedures mentioned above were carried out with solutions adjusted to neutral reaction, since it has been shown that hydrogen ion concentrations in the acid range result in progressive loss of activity. Inactivation occurs rapidly at pH 5 and below.

Qualitative Chemical Tests.—The purified material in concentrated solution gives negative biuret and Millon tests. These tests have been done directly on dry material with negative results. The Dische diphenylamine reaction for desoxyribonucleic acid is strongly positive. The orcinol test (Bial) for ribonucleic acid is weakly positive. However, it has been found that in similar concentrations pure preparations of desoxyribonucleic acid of animal origin prepared by different methods give a Bial reaction of corresponding intensity.

Although no specific tests for the presence of lipid in the purified material have been made, it has been found that crude material can be repeatedly extracted with alcohol and ether at -12°C. without loss of activity. In addition, as will be noted in the preparative procedures, repeated alcohol precipitation and treatment with chloroform result in no decrease in biological activity.

*Elementary Chemical Analysis.*¹—Four purified preparations were analyzed for content of nitrogen, phosphorus, carbon, and hydrogen. The

results are presented in Table I. The nitrogen-phosphorus ratios vary from 1.58 to 1.75 with an average value of 1.67 which is in close agreement with

TABLE I

Elementary Chemical Analysis of Purified Preparations of the Transforming Substance

Preparation No.	Carbon per cent	Hydrogen per cent	Nitrogen per cent	Phosphorus per cent	N/P ratio
37	34.27	3.89	14.21	8.57	1.66
38B	—	—	15.93	9.09	1.75
42	35.50	3.76	15.36	9.04	1.69
44	—	—	13.40	8.45	1.58
Theory for sodium desoxyribonucleate	34.20	3.21	15.32	9.05	1.69

that calculated on the basis of the theoretical structure of sodium desoxyribonucleate (tetranucleotide). The analytical figures by themselves do not establish that the substance isolated is a pure chemical entity. However, on the basis of the nitrogen-phosphorus ratio, it would appear that little protein or other substances containing nitrogen or phosphorus are present as impurities since if they were this ratio would be considerably altered.

Enzymatic Analysis.—Various crude and crystalline enzymes² have been tested for their capacity to destroy the biological activity of potent bacterial extracts. Extracts buffered at the optimal pH, to which were added crystalline trypsin and chymotrypsin or combinations of both, suffered no loss in activity following treatment with these enzymes. Pepsin could not be tested because extracts are rapidly

inactivated at the low pH required for its use. Prolonged treatment with crystalline ribonuclease under optimal conditions caused no demonstrable decrease in transforming activity. The fact that trypsin, chymotrypsin, and ribonuclease had no effect on the transforming principle is further evidence that this substance is not ribonucleic acid or a protein susceptible to the action of tryptic enzymes.

In addition to the crystalline enzymes, sera and preparations of enzymes obtained from the organs of various animals were tested to determine their effect on transforming activity. Certain of these were found to be capable of completely destroying biological activity. The various enzyme preparations tested included highly active phosphatases obtained from rabbit bone by the method of Martland and Robison (15) and from swine kidney as described by H. and E. Albers (16). In addition, a preparation made from the intestinal mucosa of dogs by Levene and Dillon (17) and containing a polynucleotidase for thymus nucleic acid was used. Pneumococcal autolysates and a commercial preparation of pancreatin were also

¹ The elementary chemical analyses were made by Dr. A. Elek of The Rockefeller Institute.

² The authors are indebted to Dr. John H. Northrop and Dr. M. Kunitz of The Rockefeller Institute for Medical Research, Princeton, N. J., for the samples of crystalline trypsin, chymotrypsin, and ribonuclease used in this work.

tested. The alkaline phosphatase activity of these preparations was determined by their action on β -glycerophosphate and phenyl phosphate, and the esterase activity by their capacity to split tributyrin. Since the highly purified transforming material isolated from pneumococcal extracts was found to contain desoxyribonucleic acid, these same enzymes were tested for depolymerase activity on known samples of desoxyribonucleic acid isolated by Mirsky³ from fish sperm and mammalian tissues. The results are summarized in Table II in which the phosphatase, esterase, and nucleodepolymerase activity of these enzymes

is compared with their capacity to destroy the transforming principle. Analysis of these results shows that irrespective of the presence of phosphatase or esterase only those preparations shown to contain an enzyme capable of depolymerizing authentic samples of desoxyribonucleic acid were found to inactivate the transforming principle.

Greenstein and Jenrette (18) have shown that tissue extracts, as well as the milk and serum of several mammalian species, contain an enzyme system which causes depolymerization of desoxyribonucleic acid. To this enzyme system Greenstein has later

TABLE II

The Inactivation of Transforming Principle by Crude Enzyme Preparations

Crude enzyme preparations	Enzymatic activity			
	Phosphatase	Tributyrin esterase	Depolymerase for desoxyribonucleate	Inactivation of transforming principle
Dog intestinal mucosa	+	+	+	+
Rabbit bone phosphatase	+	+	—	—
Swine kidney phosphatase	+	—	—	—
Pneumococcus autolysates	—	+	+	+
Normal dog and rabbit serum	+	+	+	+

given the name desoxyribonucleodepolymerase (19). These investigators determined depolymerase activity by following the reduction in viscosity of solutions of sodium desoxyribonucleate. The nucleate and enzyme were mixed in the viscosimeter and viscosity measurements made at intervals during incubation at 30°C. In the present study this method was used in the measurement of depolymerase activity except that incubation was carried out

at 37°C. and, in addition to the reduction of viscosity, the action of the enzyme was further tested by the progressive decrease in acid precipitability of the nucleate during enzymatic breakdown.

The effect of fresh normal dog and rabbit serum on the activity of the transforming substance is shown in the following experiment.

Sera obtained from a normal dog and normal rabbit were diluted with an equal volume of physiological saline. The diluted serum was divided into three equal portions. One part was heated at 65°C. for 30 minutes, another at 60°C. for 30

³ The authors express their thanks to Dr. A. E. Mirsky of the Hospital of The Rockefeller Institute for these preparations of desoxyribonucleic acid.

minutes, and the third was used unheated as control. A partially purified preparation of transforming material which had previously been dried in the lyophile apparatus was dissolved in saline in a concentration of 3.7 mg. per cc. 1.0 cc. of this solution was mixed with 0.5 cc. of the various samples of heated and unheated diluted sera, and the mixtures at pH 7.4 were incubated at 37°C. for 2 hours. After the serum had been allowed to act on the transforming material for this period, all tubes were heated at 65°C. for 30 minutes to stop enzymatic action. Serial dilutions were then made in saline and tested in triplicate for transforming activity according to the procedure described under Method of titration. The results given in Table III illustrate the differential heat inactivation of the enzymes in dog and rabbit serum which destroy the transforming principle.

From the data presented in Table III it is evident that both dog and rabbit serum in the unheated state are capable of completely destroying transforming activity. On the other hand, when samples of dog serum which have been heated either at 60°C. or at 65°C. for 30 minutes are used, there is no loss of transforming activity. Thus, in this species the serum enzyme responsible for destruction of the transforming principle is completely inactivated at 60°C. In contrast to these results, exposure to 65°C. for 30 minutes was required for complete destruction of the corresponding enzyme in rabbit serum.

The same samples of dog and rabbit serum used in the preceding experiment were also tested for their depolymerase activity on a preparation of sodium desoxyribonucleate isolated by Mirsky from shad sperm.

A highly viscous solution of the nucleate in distilled water in a concentration of 1 mg. per cc. was used. 1.0 cc. amounts of heated and unheated sera diluted in saline as shown in the preced-

ing protocol were mixed in Ostwald viscosimeters with 4.0 cc. of the aqueous solution of the nucleate. Determinations of viscosity were made immediately and at intervals over a period of 24 hours during incubation at 37°C.

The results of this experiment are graphically presented in Chart 1. In the case of unheated serum of both dog and rabbit, the viscosity fell to that of water in 5 to 7 hours. Dog serum heated at 60°C. for 30 minutes brought about no significant reduction in viscosity after 22 hours. On the

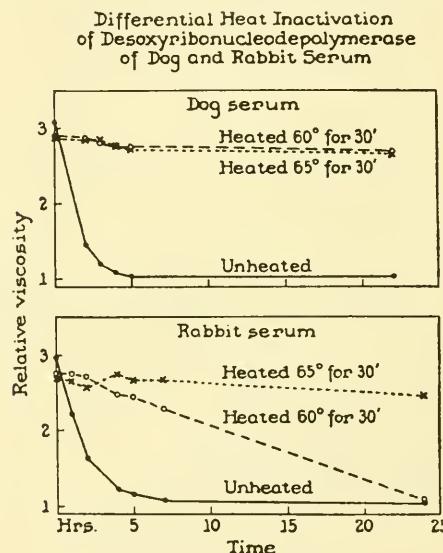


CHART 1

other hand, heating rabbit serum at 60°C. merely reduced the rate of depolymerase action, and after 24 hours the viscosity was brought to the same level as with the unheated serum. Heating at 65°C., however, completely destroyed the rabbit serum depolymerase.

Thus, in the case of dog and rabbit sera there is a striking parallelism between the temperature of inactivation of the depolymerase and that of the enzyme which destroys the activity of

TABLE III

Differential Heat Inactivation of Enzymes in Dog and Rabbit Serum Which Destroy the Transforming Substance

	Heat treatment of serum	Dilution *	TRIPPLICATE TESTS					
			1		2		3	
			Diffuse growth	Colony form	Diffuse growth	Colony form	Diffuse growth	Colony form
Dog serum	Unheated	Undiluted	—	R only	—	R only	—	R only
		1:5	—	R "	—	R "	—	R "
		1:25	—	R "	—	R "	—	R "
	60°C. for 30 min.	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII
	65°C. for 30 min.	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII
Rabbit serum	Unheated	Undiluted	—	R only	—	R only	—	R only
		1:5	—	R "	—	R "	—	R "
		1:25	—	R "	—	R "	—	R "
	60°C. for 30 min.	Undiluted	—	R only	—	R only	—	R only
		1:5	—	R "	—	R "	—	R "
		1:25	—	R "	—	R "	—	R "
	65°C. for 30 min.	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII
Control (no serum)	None	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII

* Dilution of the digest mixture of serum and transforming substance.

the transforming principle. The fact that this difference in temperature of inactivation is not merely a general property of all enzymes in the sera is evident from experiments on the heat inactivation of tributyrin esterase in the same samples of serum. In the latter instance, the results are the reverse of those observed with depolymerase since the esterase of rabbit serum is almost completely inactivated at 60°C. while that in dog serum is only slightly

affected by exposure to this temperature.

Of a number of substances tested for their capacity to inhibit the action of the enzyme known to destroy the transforming principle, only sodium fluoride has been found to have a significant inhibitory effect. Regardless of whether this enzyme is derived from pneumococcal cells, dog intestinal mucosa, pancreatin, or normal sera its activity is inhibited by fluoride.

Similarly it has been found that fluoride in the same concentration also inhibits the enzymatic depolymerization of desoxyribonucleic acid.

The fact that transforming activity is destroyed only by those preparations containing depolymerase for desoxyribonucleic acid and the further fact that in both instances the enzymes concerned are inactivated at the same temperature and inhibited by fluoride provide additional evidence for the belief that the active principle is a nucleic acid of the desoxyribose type.

Serological Analysis.—In the course of chemical isolation of the active material it was found that as crude extracts were purified, their serological activity in Type III antiserum progressively decreased without corresponding loss in biological activity. Solutions of the highly purified substance itself gave only faint trace reactions in precipitin tests with high titer Type III antipneumococcus rabbit serum.⁴ It is well known that pneumococcal protein can be detected by serological methods in dilutions as high as 1:50,000 and the capsular as well as the somatic polysaccharide in dilutions of at least 1:5,000,000. In view of these facts, the loss of serological reactivity indicates that these cell constituents have been almost completely removed from the final preparations. The fact that the transforming substance in purified state exhibits little or no serological reactivity is in striking contrast to its biological specificity in inducing pneumococcal transformation.

*Physicochemical Studies.*⁵—A purified and active preparation of the transforming substance (preparation 44) was examined in the analytical

ultracentrifuge. The material gave a single and unusually sharp boundary indicating that the substance was homogeneous and that the molecules were uniform in size and very asymmetric. Biological activity was found to be sedimented at the same rate as the optically observed boundary, showing that activity could not be due to the presence of an entity much different in size. The molecular weight cannot be accurately determined until measurements of the diffusion constant and partial specific volume have been made. However, Tennent and Vilbrandt (20) have determined the diffusion constant of several preparations of thymus nucleic acid the sedimentation rate of which is in close agreement with the values observed in the present study. Assuming that the asymmetry of the molecules is the same in both instances, it is estimated that the molecular weight of the pneumococcal preparation is of the order of 500,000.

Examination of the same active preparation was carried out by electrophoresis in the Tiselius apparatus and revealed only a single electrophoretic component of relatively high mobility comparable to that of a nucleic acid. Transforming activity was associated with the fast moving component giving the optically visible boundary. Thus in both the electrical and centrifugal fields, the behavior of the purified substance is consistent with the concept that biological activity is a property of the highly polymerized nucleic acid.

Ultraviolet absorption curves

⁴ The Type III antipneumococcus rabbit serum employed in this study was furnished through the courtesy of Dr. Jules T. Freund, Bureau of Laboratories, Department of Health, City of New York.

⁵ Studies on sedimentation in the ultracentrifuge were carried out by Dr. A. Rothen; the electrophoretic analyses were made by Dr. T. Sheldovsky, and the ultra-violet absorption curves by Dr. G. I. Lavin. The authors gratefully acknowledge their indebtedness to these members of the staff of The Rockefeller Institute.

showed maxima in the region of 2600 Å and minima in the region of 2350 Å. These findings are characteristic of nucleic acids.

Quantitative Determination of Biological Activity.—In its highly purified state the material as isolated has been found to be capable of inducing trans-

formation in amounts ranging from 0.02 to 0.003 µg. Preparation 44, the purification of which was carried out at low temperature and which had a nitrogen-phosphorus ratio of 1.58, exhibited high transforming activity. Titration of the activity of this preparation is given in Table IV.

TABLE IV
Titration of Transforming Acitivity of Preparation 44

Transforming principle Preparation 44*		Quadruplicate tests							
		1		2		3		4	
Dilution	Amount added	Diffuse growth	Colony form	Diffuse growth	Colony form	Diffuse growth	Colony form	Diffuse growth	Colony form
10 ⁻²	1.0 µg.	+	SIII	+	SIII	+	SIII	+	SIII
10 ^{-2.5}	0.3	+	SIII	+	SIII	+	SIII	+	SIII
10 ⁻³	0.1	+	SIII	+	SIII	+	SIII	+	SIII
10 ^{-3.5}	0.03	+	SIII	+	SIII	+	SIII	+	SIII
10 ⁻⁴	0.01	+	SIII	+	SIII	+	SIII	+	SIII
10 ^{-4.5}	0.003	—	R only	+	SIII	—	R only	+	SIII
10 ⁻⁵	0.001	—	R “	—	R only	—	R “	—	R “
Control	None	—	R “	—	R “	—	R “	—	R “

* Solution from which dilutions were made contained 0.5 mg. per cc. of purified material. 0.2 cc. of each dilution added to quadruplicate tubes containing 2.0 cc. of standard serum broth. 0.05 cc. of a 10⁻⁴ dilution of a blood broth culture of R36A is added to each tube.

A solution containing 0.5 mg. per cc. was serially diluted as shown in the protocol. 0.2 cc. of each of these dilutions was added to quadruplicate tubes containing 2.0 cc. of standard serum broth. All tubes were then inoculated with 0.05 cc. of a 10⁻⁴ dilution of a 5 to 8 hour blood broth culture of R36A. Transforming activity was determined by the procedure described under Method of titration.

The data presented in Table IV show that on the basis of dry weight 0.003 µg. of the active material brought about transformation. Since the reaction system containing the 0.003 µg. has a volume of 2.25 cc., this represents a final concentration of the purified substance of 1 part in 600,000,000.

DISCUSSION

The present study deals with the results of an attempt to determine the chemical nature of the substance inducing specific transformation of pneumococcal types. A desoxyribonucleic acid fraction has been isolated from Type III pneumococci which is capable of transforming unencapsulated R variants derived from *Pneumococcus Type II* into fully encapsulated Type III cells. Thompson and Dubos (21) have isolated from pneumococci a nucleic acid of the ribose type. So far as the writers are aware, however, a nucleic acid of the desoxyribose type has not heretofore been recovered from pneumococci nor has

specific transformation been experimentally induced *in vitro* by a chemically defined substance.

Although the observations are limited to a single example, they acquire broader significance from the work of earlier investigators who demonstrated the interconvertibility of various pneumococcal types and showed that the specificity of the changes induced is in each instance determined by the particular type of encapsulated cells used to evoke the reaction. From the point of view of the phenomenon in general, therefore, it is of special interest that in the example studied, highly purified and protein-free material consisting largely, if not exclusively, of desoxyribonucleic acid is capable of stimulating unencapsulated R variants of *Pneumococcus Type II* to produce a capsular polysaccharide identical in type specificity with that of the cells from which the inducing substance was isolated. Equally striking is the fact that the substance evoking the reaction and the capsular substance produced in response to it are chemically distinct, each belonging to a wholly different class of chemical compounds.

The inducing substance, on the basis of its chemical and physical properties, appears to be a highly polymerized and viscous form of sodium desoxyribonucleate. On the other hand, the Type III capsular substance, the synthesis of which is evoked by this transforming agent, consists chiefly of a non-nitrogenous polysaccharide constituted of glucose-glucuronic acid units linked in glycosidic union (22). The presence of the newly formed capsule containing this type-specific polysaccharide confers on the transformed cells all the distinguishing characteristics of *Pneumococcus Type III*. Thus, it is evident that the inducing substance and the substance pro-

duced in turn are chemically distinct and biologically specific in their action and that both are requisite in determining the type specificity of the cell of which they form a part.

The experimental data presented in this paper strongly suggest that nucleic acids, at least those of the desoxyribose type, possess different specificities as evidenced by the selective action of the transforming principle. Indeed, the possibility of the existence of specific differences in biological behavior of nucleic acids has previously been suggested (23, 24) but has never been experimentally demonstrated owing in part at least to the lack of suitable biological methods. The techniques used in the study of transformation appear to afford a sensitive means of testing the validity of this hypothesis, and the results thus far obtained add supporting evidence in favor of this point of view.

If it is ultimately proved beyond reasonable doubt that the transforming activity of the material described is actually an inherent property of the nucleic acid, one must still account on a chemical basis for the biological specificity of its action. At first glance, immunological methods would appear to offer the ideal means of determining the differential specificity of this group of biologically important substances. Although the constituent units and general pattern of the nucleic acid molecule have been defined, there is as yet relatively little known of the possible effect that subtle differences in molecular configuration may exert on the biological specificity of these substances. However, since nucleic acids free or combined with histones or protamines are not known to function antigenically, one would not anticipate that such differences would be revealed by immunological techniques. Consequently, it is perhaps not surpris-

ing that highly purified and protein-free preparations of deoxyribonucleic acid, although extremely active in inducing transformation, showed only faint trace reactions in precipitin tests with potent Type III antipneumococcus rabbit sera.

From these limited observations it would be unwise to draw any conclusion concerning the immunological significance of the nucleic acids until further knowledge on this phase of the problem is available. Recent observations by Lackman and his collaborators (25) have shown that nucleic acids of both the yeast and thymus type derived from hemolytic streptococci and from animal and plant sources precipitate with certain antipneumococcal sera. The reactions varied with different lots of immune serum and occurred more frequently in antipneumococcal horse serum than in corresponding sera of immune rabbits. The irregularity and broad cross reactions encountered led these investigators to express some doubt as to the immunological significance of the results. Unless special immunochemical methods can be devised similar to those so successfully used in demonstrating the serological specificity of simple non-antigenic substances, it appears that the techniques employed in the study of transformation are the only ones available at the present for testing possible differences in the biological behavior of nucleic acids.

Admittedly there are many phases of the problem of transformation that require further study and many questions that remain unanswered largely because of technical difficulties. For example, it would be of interest to know the relation between rate of reaction and concentration of the transforming substance; the proportion of cells transformed to those that remain unaffected in the reaction system.

However, from a bacteriological point of view, numerical estimations based on colony counts might prove more misleading than enlightening because of the aggregation and sedimentation of the R cells agglutinated by the anti-serum in the medium. Attempts to induce transformation in suspensions of resting cells held under conditions inhibiting growth and multiplication have thus far proved unsuccessful, and it seems probable that transformation occurs only during active reproduction of the cells. Important in this connection is the fact that the R cells, as well as those that have undergone transformation, presumably also all other variants and types of pneumococci, contain an intracellular enzyme which is released during autolysis and in the free state is capable of rapidly and completely destroying the activity of the transforming agent. It would appear, therefore, that during the logarithmic phase of growth when cell division is most active and autolysis least apparent, the cultural conditions are optimal for the maintenance of the balance between maximal reactivity of the R cell and minimal destruction of the transforming agent through the release of autolytic ferments.

In the present state of knowledge any interpretation of the mechanism involved in transformation must of necessity be purely theoretical. The biochemical events underlying the phenomenon suggest that the transforming principle interacts with the R cell giving rise to a coordinated series of enzymatic reactions that culminate in the synthesis of the Type III capsular antigen. The experimental findings have clearly demonstrated that the induced alterations are not random changes but are predictable, always corresponding in type specificity to that of the encapsulated cells from

which the transforming substance was isolated. Once transformation has occurred, the newly acquired characteristics are thereafter transmitted in series through innumerable transfers in artificial media without any further addition of the transforming agent. Moreover, from the transformed cells themselves, a substance of identical activity can again be recovered in amounts far in excess of that originally added to induce the change. It is evident, therefore, that not only is the capsular material reproduced in successive generations but that the primary factor, which controls the occurrence and specificity of capsular development, is also reduplicated in the daughter cells. The induced changes are not temporary modifications but are permanent alterations which persist provided the cultural conditions are favorable for the maintenance of capsule formation. The transformed cells can be readily distinguished from the parent R forms not alone by serological reactions but by the presence of a newly formed and visible capsule which is the immunological unit of type specificity and the accessory structure essential in determining the infective capacity of the microorganism in the animal body.

It is particularly significant in the case of pneumococci that the experimentally induced alterations are definitely correlated with the development of a new morphological structure and the consequent acquisition of new antigenic and invasive properties. Equally if not more significant is the fact that these changes are predictable, type-specific, and heritable.

Various hypotheses have been advanced in explanation of the nature of the changes induced. In his original description of the phenomenon Griffith (1) suggested that the dead bacteria in the inoculum might furnish

some specific protein that serves as a "pabulum" and enables the R form to manufacture a capsular carbohydrate.

More recently the phenomenon has been interpreted from a genetic point of view (26, 27). The inducing substance has been likened to a gene, and the capsular antigen which is produced in response to it has been regarded as a gene product. In discussing the phenomenon of transformation Dobzhansky (27) has stated that "If this transformation is described as a genetic mutation—and it is difficult to avoid so describing it—we are dealing with authentic cases of induction of specific mutations by specific treatments. . . ."

Another interpretation of the phenomenon has been suggested by Stanley (28) who has drawn the analogy between the activity of the transforming agent and that of a virus. On the other hand, Murphy (29) has compared the causative agents of fowl tumors with the transforming principle of *Pneumococcus*. He has suggested that both these groups of agents be termed "transmissible mutagens" in order to differentiate them from the virus group. Whatever may prove to be the correct interpretation, these differences in viewpoint indicate the implications of the phenomenon of transformation in relation to similar problems in the fields of genetics, virology, and cancer research.

It is, of course, possible that the biological activity of the substance described is not an inherent property of the nucleic acid but is due to minute amounts of some other substance adsorbed to it or so intimately associated with it as to escape detection. If, however, the biologically active substance isolated in highly purified form as the sodium salt of desoxyribonucleic acid actually proves to be the transforming principle, as the available evidence strongly suggests, then nucleic

acids of this type must be regarded not merely as structurally important but as functionally active in determining the biochemical activities and specific characteristics of pneumococcal cells. Assuming that the sodium desoxyribonucleate and the active principle are one and the same substance, then the transformation described represents a change that is chemically induced and specifically directed by a known chemical compound. If the results of the present study on the chemical nature of the transforming principle are confirmed, then nucleic acids must be regarded as possessing biological specificity the chemical basis of which is as yet undetermined.

SUMMARY

1. From Type III pneumococci a biologically active fraction has been isolated in highly purified form which in exceedingly minute amounts is capable under appropriate cultural conditions of inducing the transformation of unencapsulated R variants of *Pneumococcus Type II* into fully encapsulated cells of the same specific type as that of the heat-killed microorganisms from which the inducing material was recovered.

2. Methods for the isolation and purification of the active transforming material are described.

3. The data obtained by chemical, enzymatic, and serological analyses together with the results of preliminary studies by electrophoresis, ultracentrifugation, and ultraviolet spectroscopy indicate that, within the limits of the methods, the active fraction contains no demonstrable protein, unbound lipid, or serologically reactive polysaccharide and consists principally, if not solely, of a highly polymerized, viscous form of desoxyribonucleic acid.

4. Evidence is presented that the chemically induced alterations in cellular structure and function are predictable, type-specific, and transmissible in series. The various hypotheses that have been advanced concerning the nature of these changes are reviewed.

CONCLUSION

The evidence presented supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of *Pneumococcus Type III*.

BIBLIOGRAPHY

1. Griffith, F., *J. Hyg.*, Cambridge, Eng., 1928, 27:113.
2. Neufeld, F., and Levinthal, W., *Z. Immunitätsforsch.*, 1928, 55:324.
3. Baurhenn, W., *Centr. Bakt.*, 1. Abt., Orig., 1932, 126:68.
4. Dawson, M. H., *J. Exp. Med.*, 1930, 51: 123.
5. Dawson, M. H., and Sia, R. H. P., *J. Exp. Med.*, 1931, 54:681.
6. Alloway, J. L., *J. Exp. Med.*, 1932, 55:91; 1933, 57:265.
7. Berry, G. P., and Dedrick, H. M., *J. Bact.*, 1936, 31:50.
8. Berry, G. P., *Arch. Path.*, 1937, 24:533.
9. Hurst, E. W., *Brit. J. Exp. Path.*, 1937, 18:23. Hoffstadt, R. E., and Pilcher, K. S., *J. Infect. Dis.*, 1941, 68:67. Gardner, R. E., and Hyde, R. R., *J. Infect. Dis.*, 1942, 71:47. Houlahan, R. B., *Proc. Soc. Exp. Biol. and Med.*, 1942, 51:259.
10. MacLeod, C. M., and Mirick, G. S., *J. Bact.*, 1942, 44:277.
11. Dawson, M. H., *J. Exp. Med.*, 1928, 47: 577; 1930, 51:99.
12. Sevag, M. G., *Biochem. Z.*, 1934, 273:419. Sevag, M. G., Lackman, D. B., and Smolens, J., *J. Biol. Chem.*, 1938, 124: 425.
13. Dubos, R. J., and Avery, O. T., *J. Exp. Med.*, 1931, 54:51. Dubos, R. J., and Bauer, J. H., *J. Exp. Med.*, 1935, 62:271.
14. Liu, S., and Wu, H., *Chinese J. Physiol.*, 1938, 13:449.
15. Martland, M., and Robison, R., *Biochem. J.*, 1929, 23:237.

16. Albers, H., and Albers, E., *Z. physiol. Chem.*, 1935, 232:189.
17. Levene, P. A., and Dillon, R. T., *J. Biol. Chem.*, 1933, 96:461.
18. Greenstein, J. P., and Jenrette, W. Y., *J. Nat. Cancer Inst.*, 1940, 1:845.
19. Greenstein, J. P., *J. Nat. Cancer Inst.*, 1943, 4:55.
20. Tennent, H. G., and Vilbrandt, C. F., *J. Am. Chem. Soc.*, 1943, 65:424.
21. Thompson, R. H. S., and Dubos, R. J., *J. Biol. Chem.*, 1938, 125:65.
22. Reeves, R. E., and Goebel, W. F., *Biol. Chem.*, 1941, 139:511.
23. Schultz, J., in *Genes and chromosomes. Structure and organization*, *Cold Spring Harbor symposia on quantitative biology*, Cold Spring Harbor, Long Island Biological Association, 1941, 9:55.
24. Mirsky, A. E., in *Advances in enzymology and related subjects of biochemistry*, (F. F. Nord and C. H. Werkman, editors), New York, Interscience Publishers, Inc., 1943, 3:1.
25. Lackman, D., Mudd, S., Sevag, M. G., Smolens, J., and Wiener, M., *J. Immunol.*, 1941, 40:1.
26. Gortner, R. A., *Outlines of biochemistry*, New York, Wiley, 2nd edition, 1938, 547.
27. Dobzhansky, T., *Genetics and the origin of the species*, New York, Columbia University Press, 1941, 47.
28. Stanley, W. M., in Doerr, R., and Hal-lauer, C., *Handbuch der Virusforschung*, Vienna, Julius Springer, 1938, 1:491.
29. Murphy, J. B., *Tr. Assn. Am. Physn.*, 1931, 46:182; *Bull. Johns Hopkins Hosp.*, 1935, 56:1.



Gene Recombination in *Escherichia Coli*

JOSHUA LEDERBERG and EDWARD L. TATUM

Reprinted by authors' and publisher's permission from *Nature*, vol. 158, 1946, p. 558.

In selecting papers for this volume I have deliberately omitted most of the papers published prior to 1952 in the highly specialized field of study known as "microbial genetics," because of the availability of the book "Papers in Microbial Genetics," edited by J. Lederberg and published by the University of Wisconsin Press. That book serves the same function for that area of genetics as does this volume for much of the rest of the field.

Omitted from that volume, however, is this short note on the occurrence of sex in a bacterium. It is of far-reaching importance in that it is the initial announcement of an entirely new and unsuspected phenomenon. The important role of sex in hereditary and evolutionary processes has been evident from the time of publication of the first paper in this series by Mendel, and has been repeatedly emphasized by Sutton, Bridges, Sturtevant, and Morgan, among

others. Sex plays its greatest role in heredity in that it provides for the recombination of genes into new, "experimental" series, which make up the raw material upon which natural selection acts. Lederberg and Tatum reverse the original procedure in that they utilize the phenomenon of recombination to demonstrate the existence of sex in bacteria. Since bacteria reproduce rapidly and abundantly, the discovery of sexual processes in their reproduction made them as important and valuable as a research tool as *Drosophila* and *Neurospora*. A considerable series of papers followed this original announcement, and the interested reader can find many of them in the book cited above.

ANALYSIS OF MIXED CULTURES OF NUTRITIONAL MUTANTS

The mutants consist of strains which differ from their parent wild type, strain K-12, in lacking the ability to synthesize growth-factors. As a result of these deficiencies they will only grow in media supplemented with their specific nutritional requirements. In these mutants single nutritional requirements are established as single mutational steps under the influence of X-ray or ultra-violet.^{1,2} By successive treatments, strains with several requirements have been obtained.

In the recombination studies here reported, two triple mutants have been used: Y-10, requiring threonine, leucine and thiamin, and Y-24, requiring biotin, phenylalanine and cystine. These strains were grown in mixed culture in 'Bacto' yeast-beef broth. When fully grown, the cells were washed with sterile water and inoculated heavily into synthetic agar medium, to which various supplements had been added to allow the growth

of colonies of various nutritional types. This procedure readily allows the detection of very small numbers of cell types different from the parental forms.

The only new types found in 'pure' cultures of the individual mutants were occasional forms which had reverted for a single factor, giving strains which required only two of the original three substances. In mixed cultures, however, a variety of types has been found. These include wild-type strains with no growth-factor deficiencies and single mutant types requiring only thiamin or phenylalanine. In addition, double requirement types have been obtained, including strains deficient in the syntheses of biotin and leucine, biotin and threonine, and biotin and thiamin respectively. The wild-type strains have been studied most intensively, and several independent lines of evidence have indicated their stability and homogeneity.

In other experiments, using the triple mutants mentioned, except that one was resistant to the coli phage T1 (obtained by the procedure of Luria and Delbrück³), nutritionally wild-type strains were found both in sensitive and in resistant categories. Similarly, recombinations between bio-

¹ Tatum, E. L., *Proc. Nat. Acad. Sci.*, 31: 215, 1945.

² Tatum, E. L., *Cold Spring Harbor Symposia Quant. Biol.*, vol. 11, (in the press, 1946).

³ Luria, S. E. and Delbrück, M., *Genetics*, 28:491, 1943.

chemical requirements and phage resistance have frequently been found.

These types can most reasonably be interpreted as instances of the assortment of genes in new combinations. In order that various genes may have the opportunity to recombine, a cell fusion would be required. The only apparent alternative to this interpretation would be the occurrence in the medium of transforming factors capable of inducing the mutations of genes, bilaterally, both to and from the wild condition. Attempts at the induction of transformations in single

cultures by the use of sterile filtrates have been unsuccessful.

The fusion presumably occurs only rarely, since in the cultures investigated only one cell in a million can be classified as a recombination type. The hypothetical zygote has not been detected cytologically.

These experiments imply the occurrence of a sexual process in the bacterium *Escherichia coli*; they will be reported in more detail elsewhere.

This work was supported in part by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.



Genetic Effects of the Atomic Bombs in Hiroshima and Nagasaki

Genetics Conference, Committee on Atomic Casualties,
National Research Council

Reprinted by publisher's permission from *Science*, vol. 106, 1947, pp. 331-333.

Up to this point, many of the papers that have been included have already demonstrated their significance by the ways in which they have affected the directions and aims of the field of genetics. They have created new problems, pointed out new pathways, and provided new methods that proved fruitful in genetic analysis and understanding. Obviously, it is difficult to select such papers from the literature of the past few years, because a certain amount of time is always necessary to permit a paper to reveal its basic importance.

This paper and the one by Sturtevant (p. 259) strongly indicate the existence of still another aspect of genetics, the significance of which lies in the relationship of man to mankind. The geneticist has suddenly found himself in possession of information of the utmost importance in our modern world, and at the same time finds himself painfully lacking in information most urgently needed for

decisions of far-reaching social significance. Biologists, for the most part, lead rather sheltered lives. Their discoveries are of interest principally to their immediate colleagues, although commercial aspects of many biological discoveries have become increasingly important. This is no longer true of the geneticist. His activities are of public interest; his debates are reported in the world's press. His discoveries are widely publicized, and are often misinterpreted and garbled in the transmission. He has moved, willy-nilly, into the public eye. The consequences to society of discoveries that are likely to affect the human genetic constitution must be investigated and determined, and the new trend for genetic research is clear-cut.

The first of these two papers illustrates another general biological phenomenon, that of team research. This is the report of a group of research workers, not of a single man. Research today in any area is likely to demand too much specialized knowledge and background for any individual to encompass, and as a consequence several specialists cooperate to bring their pooled knowledge to bear on a problem.

This paper introduces the problem of the effects of the atomic bomb on the genetics of mankind. It is perhaps one of the most active and most heavily supported areas of research today.

The Atomic Energy Commission recently formally signified its intention of supporting long-range medical studies of the survivors of the atomic bombings in Japan, to be conducted by the Committee on Atomic Casualties of the National Research Council. One aspect of these studies will concern the much-discussed potential genetic effects of the bombs. The background of this program begins shortly after Japan's surrender, when a Joint Army-Navy Commission made extensive observations in Hiroshima and Nagasaki on the survivors of the bombings. At the conclusion of the Commission's work its chairman, Col. A. W. Oughterson, M.C., AUS, recommended to the Surgeon General of the Army that the Council be requested to undertake a long-range study of the medical and biological effects of the atomic bomb, and this recommendation was transmitted by Surgeon Gen. Norman T. Kirk to Lewis H. Weed, chairman of the Division of Medical Sciences. As a result, in June 1946 a conference group was convened by the Council, and in November, following its recommenda-

tion, a five-man commission composed of representatives of the Council, the Army, and the Navy left for Japan for the purpose of determining the current status of Japanese work on atomic bomb casualties, evaluating the feasibility of American participation in continued research on these casualties, and indicating the lines along which such studies should proceed. This commission, known as the Atomic Bomb Casualty Commission and composed of Austin Brues, Paul S. Henshaw, Lt. Melvin Block, M.C., AUS, Lt. James V. Neel, M.C., AUS, and Lt (j.g.) Frederick Ullrich, (MC) USNR, submitted a report of its findings to the Council in January 1947.

The June 1946 conference group had recommended that appropriate action be taken to obtain a Presidential Directive authorizing the National Research Council to initiate a long range study of the atomic bomb effects. This Directive was issued at the request of the Secretary of the Navy, James T. Forrestal, in November 1946, and on its authority the Council, in January 1947, established a Committee on Atomic Casualties, composed

of Thomas M. Rivers (chairman), George W. Beadle, Detlev W. Bronk, Austin Brues, George M. Lyon, C. P. Rhoads, Shields Warren, Stafford L. Warren, George H. Whipple, and Raymond E. Zirkle.

The potential genetic effects of the atomic bomb were apparent to all interested students from the day the first bomb was dropped—in fact, to some, well before that time. A consideration of genetic studies was one facet of the work of the Atomic Bomb Casualty Commission, and a section of its January 1947 report was devoted to this subject. This phase of the work was to a large extent the responsibility of Lt. James V. Neel.

On June 24, 1947, the Committee on Atomic Casualties arranged a conference on the potential genetic effects of the atomic bombs. At this meeting, which was attended by George W. Beadle (chairman), Donald R. Charles, Charles H. Danforth, Herman J. Muller, Laurence H. Snyder, and Lt. Neel, the latter submitted a report of preliminary genetic studies, based on his observations in Japan during the preceding six months. Following a thorough appraisal of the problem, the conference voted to recommend to the Committee on Atomic Casualties that a program be undertaken in Japan along the lines sketched out in the Neel report. This recommendation was accepted at a meeting of the Committee on June 26, 1947. The conference also recommended that a statement be prepared, briefly summarizing the current status of the problem. This statement follows.

WHETHER THE ATOMIC BOMBS dropped on Hiroshima and Nagasaki will have detectable genetic effects on the Japanese is a question of widespread interest. The purpose of the present note is to show briefly that (1) many difficulties beset any attempt to obtain a valid answer to this question and (2) even after a long-term study, such as that outlined below, it still may not be possible to determine just how much genetic damage was done at Hiroshima and Nagasaki.

This memorandum is essentially a partial summary of the material presented by Lt. James V. Neel at the meeting of the Conference on Genetics convened by the Committee on Atomic Casualties of the National Research Council on June 24, 1947, but with certain additional considerations which grew out of the deliberations of the Conference.

It must first be recognized that, inasmuch as the majority of mutations occurring in animals are recessive, only the relatively small proportion of mutations which are dominants may be expected to show effects in the first postbomb generation. The potential range in their effects is very wide. Dominant mutations with large, clear-cut manifestations can be expected to be much rarer than those with smaller, but possibly quite significant, effects on bodily dimensions, life span, etc. But the detection of these latter is a matter of great difficulty with present techniques. For practical considerations investigation will have to be concentrated chiefly on the class with such large effects as may lead to stillbirths, to live births with gross external abnormality, or to internal defects causing death or serious illness in infancy.

Since there is no general agreement as to what proportion of cases of abnormal fetal development is genetically determined, and what proportion is due to nongenetic factors, an increased incidence of morphologically abnormal fetuses following irradiation may not be used as an index of the frequency of genetic change until the nongenetic effects of this irradiation on the reproductive history of the mother have been determined. This point will be very difficult to evaluate.

It is obvious that in this case the approach to the problem of genetic effects is the statistical one. It is un-

likely that any individual and specific pathology in a post-irradiation generation can ever be attributed with certainty to the effects of the bomb, but if there is a definite increase in the occurrence of abortions, miscarriages, stillbirths, and abnormal products of conception, one may surmise that this is related to the bombing—although some of the effects need not necessarily be genetic. Appropriate control studies in other Japanese cities are therefore of the utmost importance.

The survivors of the bombing received amounts of irradiation ranging from negligible to just short of lethal. It is impossible to say on a priori grounds whether an adequate number of people absorbed sufficient radiation to produce enough dominant mutations to result in detectable effects. However, comparison of this situation with the experimental data on infra-human material suggests that these effects, if detectable, will be small. The median lethal dose for whole body irradiation in man is probably in the neighborhood of 500 Roentgen units (Shields Warren. *Physiol. Rev.*, 1944, 24:225-238). It is likely that many individuals in Hiroshima and Nagasaki who received high but sub-lethal doses of irradiation sustained other injuries which, in combination with the radiation damage, resulted in death. Thus, the mean radiation dose received by the surviving population will be lower than expected from a consideration of the median lethal dose alone. In terms of radiation genetics this is a small exposure, expected from data on animals to produce a frequency of dominant mutations which would increase the normal frequency of abnormal offspring by so small a proportion as to be difficult to demonstrate. Large-scale studies, utilizing all available material, plus accurate vital

statistics, are thus necessary if data of value are to be obtained. In this connection it is important to bear in mind that the available children born to parents who received significant amounts of irradiation will probably not exceed 12,000 or 13,000 within the next 10 years.

The Japanese had recognized the importance of genetic studies and, under great difficulties, were organizing a program in Hiroshima when the Atomic Bomb Casualty Commission arrived. They had planned to compare the present and future frequency of abnormal births in Hiroshima with the frequencies reported in their medical literature and vital statistics during the prewar years. But it is by no means sure either that the prewar figures were sufficiently accurate or that the present reporting of vital statistics would be wholly effective in detecting rare effects of the atomic bomb radiations. It cannot be too strongly emphasized that there is at present absolutely no reliable evidence on which to base any opinion concerning the absolute or relative frequency of congenital abnormalities among children being born in Hiroshima and Nagasaki. Unfortunately, a good deal of misinformation is currently in circulation.

Two independent programs, one Japanese and one American, would involve needless duplication of effort. A joint undertaking is therefore indicated. In view of the fact that the Japanese are actively attempting to initiate genetic studies, it seems that any American efforts which may materialize should contemplate cooperation with the Japanese in an attempt to ensure an efficient and satisfactory program. The Neel report contains the following seven specific recommendations as to the organization of a program:

(1) Organize, in Hiroshima, Nagasaki, and a control area or areas, a modified system of pregnancy registration, this to include the irradiation history of the parents.

(2) Obtain as complete information as possible on the outcome of each registered pregnancy.

(3) Follow up each report of an abnormal termination of pregnancy or a congenital malformation with detailed family studies.

(4) Develop a system of checking on the completeness and accuracy of registration of births and deaths, such as requiring at intervals dual registration by both the family and the obstetrician or midwife.

(5) Conduct these studies on a sufficiently large scale that the results will have statistical significance.

(6) Integrate this program with a system of periodic examination of the offspring of irradiated persons and with careful death certification, so that genetic effects not apparent at birth but detected subsequently may be recorded. In particular, causes of infant mortality should be accurately recorded.

(7) Place this program in competent Japanese hands, through the Japanese Government, with only enough American supervision and cooperation, including supplies, to facilitate a successful program.

This program must extend over a period of 10-20 years before a significant amount of data can be accumulated, and quite possibly an even longer period of study, extending to the second and subsequent generations, will be indicated.

Certain practical limitations of the program may be considered at this point. The most difficult problem will be to obtain the necessary completeness of reporting. This will require constant effort, a wide educational program, and frequent cross-checks. Congenital malformations occurring within Japanese families may sometimes not be reported. This is perhaps

more likely to occur in Japan than in this country, because probably less than 10 per cent of Japanese births occur in hospitals as these are defined in the United States. To what extent stillbirths and malformations occurring outside a hospital will be recorded depends on the vigor with which the problem is pursued. It will be difficult to get evenly matched teams of investigators for bombed and control areas. Furthermore, once people living in Hiroshima and Nagasaki learn that stillbirths and malformations may possibly be attributed to the effects of the bomb, they will probably lose some of their reluctance to report such matters, whereas this will not be the case in a control area.

Japan is now a defeated and occupied country, under severe postwar stress, whose people have a very different psychology from our own. A program such as that under consideration will proceed much more slowly there than it would in this country.

In order to reduce the possibility that a negative result of the investigation on Japanese material be interpreted by the medical and lay public as meaning that important genetic effects were not produced, it is essential that a comparable effort be expended in experimentation on other mammalian material, in which genetic effects of different kinds can much more readily be brought to light. In this way it should be possible to throw light upon the proportion of the total genetic effects produced by the radiation that would have been detectable by the methods used in the investigation on the human material, and the serious danger of misinterpretation of the latter results would be minimized.

Recognizing the difficulties briefly touched upon in the foregoing paragraphs, the Conference on Genetics voted unanimously to record the fol-

lowing expression of its attitude toward the genetic program: "Although there is every reason to infer that genetic effects can be produced and have been produced in man by atomic radiation, nevertheless the conference wishes to make it clear that it cannot guarantee significant results from this or any other study on the

Japanese material. In contrast to laboratory data, this material is too much influenced by extraneous variables and too little adapted to disclosing genetic effects. In spite of these facts, the conference feels that this unique possibility for demonstrating genetic effects caused by atomic radiation should not be lost."



The Origin and Behavior of Mutable Loci in Maize

BARBARA MCCLINTOCK

Reprinted by author's and publisher's permission from *Proceedings of the National Academy of Sciences*, vol. 36, 1950, pp. 344-355.

A point that has been neglected in the introductions to these papers has been the give and take that exists between the two branches of biology, which is implicit in the study of genetics. Mendel's original work was based on a plant species. Cuenot discovered that Mendel's laws applied equally well and were readily observable in the mouse. Since this beginning the botanists and zoologists have had to keep a close eye on each other, with discoveries of genetic phenomena in one kingdom rapidly extrapolated into the other. It has long been obvious that the differences between plants and animals were not extremely significant on the level of the gene, and that genetics is properly a science of biology, requiring a knowledge of both botany and zoology.

McClintock's paper deals with a phenomenon thoroughly studied in Drosophila, which she found occurring in corn. The value of this paper lies in the fact that she was obviously not satisfied solely with the discovery of a common phenomenon between plant and animal, but then worked out the way in which the events took place. The existence of an "activator" gene, which expresses itself primarily by influencing the mutation of a second gene, was new and interesting. This paper establishes the mutagenic effect of one member of the gene complex upon another, and provides an internal as well as the more widely demonstrated and familiar external source of mutation.

IN THE COURSE OF AN EXPERIMENT designed to reveal the genic composition of the short arm of chromosome 9, a phenomenon of rare occurrence (or recognition) in maize began to appear with remarkably high frequencies in the cultures. The terms mutable genes, unstable genes, variegation, mosaicism, mutable loci or "position-effect" have been applied to this phenomenon. Its occurrence in a wide variety of organisms has been recognized. The most extensive investigations of this phenomenon have been undertaken in *Drosophila melanogaster*.¹ In this organism, the conditions associated with the origin of genic instability have been well defined. The part played by the heterochromatic materials of the chromosomes, in inducing and controlling the type of variegation and its time and frequency of occurrence, has been established. It has not been generally recognized that the instability of genic expression in other organisms may be essentially the same as that occurring in *Drosophila*.

As stated above, a large number of mutable loci have recently arisen in the maize cultures and are continuing to arise anew. The loci affect variegation for many different kinds of plant characters, each locus being concerned with a particular character or occasionally several characters. Some of these loci are *c*, *yg₂*, *wx*, *a₂*, *y*, *pyd*, which are well-investigated units in maize.² Others involve previously unknown genetic units. The same types of genic instability appearing in the maize cultures have been described in

many other organisms. The behavior of these new mutable loci in maize cannot be considered peculiar to this organism. The author believes that the mechanism underlying the phenomenon of variegation is basically the same in all organisms. The reasons for this conclusion will be made apparent in the discussion.

The initial appearance of the burst of newly arising mutable loci occurred in the progeny coming from the self-pollination of about 450 plants which had each undergone a series of events in their early development where the short arm of chromosome 9 was subjected to drastic structural modifications. These events took place during the "chromosome type" of breakage-fusion-bridge cycle.³ The modifications that this mechanism produces are: one or more duplications of segments of the short arm, deficiencies of one or more segments of various lengths, structural modifications of the heterochromatic knob substance, duplications of the knobs with or without structural modifications, and various combinations of these several types of modifications. The chromosome complement of over 150 of these plants were examined at pachytene to determine the nature of the structural modifications that had occurred. In addition to the modifications of the short arm of chromosome 9 listed above, some of the plants had other modifications, many of which are particularly significant because they involve the substances in the chromosome that are believed to be responsible for the origin and behavior of mutable loci—the heterochromatic knobs and centromeres. Altogether, 48 such structural modifications have been analyzed, most particularly in the above-mentioned plants but also in

¹ Lewis, E. B. *Advances in Genetics*, 3:73-115, 1950.

² The symbols refer to genes affecting the parts of the plant as follows: *c*, aleurone pigment; *yg₂*, chlorophyll; *wx*, composition of starch in pollen and endosperm; *a₂*, aleurone pigment; *y*, starch composition of endosperm; *pyd*, chlorophyll.

³ McClintock, B., *Proc. Natl. Acad. Sci.*, 28:458-463, 1942.

some other plants that had received a chromosome 9 with a newly broken end. Fourteen involved modifications of chromosome 9 other than those listed above (telocentric chromosomes, isochromosomes, extra chromosomes 9 with particular modifications, etc.). Four arose from fusion of the centromere of chromosome 9 with the centromere of another chromosome. Four resulted from fusion of the knob substance of the short arm of chromosome 9 with the centromere of chromosome 9. Twenty-four resulted from fusions of the knob substance of the short arm of chromosome 9 with other regions in the chromosome complement: eighteen were with other knobs or with regions very close to these knobs, four were insufficiently analyzed as to the positions of the fusion, and two did not involve a known knob region. In two cases, inversions were present in other chromosomes. The regions involved were the knob and centromere in one of these chromosomes and the nucleolus organizer and the centromere in the other chromosome. There can be no question that these "spontaneous translocations" are nonrandom with respect to the location of the breaks and fusions. The heterochromatic knob and centromere regions are mainly involved.

In the cultures arising from self-pollination of the plants that had undergone the chromosome type of breakage-fusion-bridge cycle in their early development, about 40 different mutable loci were recognized. The majority of such mutable loci could not have been present in the parents of these plants, for the stocks from which they arose had been under investigation for some years without showing evidence of the presence of such a large number of unstable loci. It was concluded, therefore, that either some part of the mechanism concerned with

the breakage-fusion-bridge cycle or some of the structural modifications resulting from it were responsible for conditions that produced this burst. That some of the mutable loci were located in or associated with chromosome 9 was realized in the first tests. Other mutable loci, on the other hand, did not show any obvious association with chromosome 9.

The mutable loci fall into two major classes: (1) those that require a separate activator factor for instability to be expressed, and (2) those that are autonomous with respect to the factor that controls the onset of mutability. They also may be subdivided on a quite different basis. This is related to the types of expression of the mutations that occur. The following types are present: (a) Changes from the mutant to, or close to, the wild-type expression. After such a mutation, the locus may be permanently stabilized. It may no longer show evidence of the instability phenomenon. (b) A second group, similar to (a) except that the mutation to wild-type does not produce stability of the locus. The wild-type-producing locus, in turn, may mutate to give the recessive expression. (c) A third type where the mutations give rise to a series of alleles of the affected loci. These alleles are distinguished by different degrees of quantitative expression of the normal phenotype. Most of these are relatively stable; only rarely does instability again appear. (d) A fourth type, similar to (c). Most of the alleles, however, are not stable for they, in turn, can mutate in the direction of a higher or lower grade of quantitative expression of the phenotype. Mutable loci showing these different types of expression of mutation are found in both the major classes, that is, in the activator-requiring class and in the autonomous class.

The accumulated observations and data from a study of a number of these mutable loci are so extensive that no short account would give sufficient information to prepare the reader for an independent judgment of the nature of the phenomenon. It is realized that this is unfortunate. Manuscripts giving full accounts of some of this phenomenon are in preparation. Since this task will require much time to fulfill, the author has decided to present this short account of the general nature of the study, and the conclusions and interpretations that have been drawn. In this account only short summaries will be given of some of the pertinent information that has led to the conclusions to be presented. These conclusions are concerned with the origin of mutable loci, the events occurring at these loci that result in a change in phenotypic expression, the reasons for changes in the frequency of visible mutations at these loci, the factors controlling the time when mutations will occur, the production of mutations at the α_1 locus in maize without Dt being present, and heterochromatin as the probable controlling factor.

A fortunate discovery was made early in the study of the mutable loci which proved to be of singular importance in showing the kinds of events that are associated with their origin and behavior. A locus was found in the short arm of chromosome 9 at which breaks were occurring in somatic cells. The time and frequency of the breakage events occurring at this Ds (Dissociation) locus appeared to be the same as the time and frequency of the mutation-producing events occurring at some of the mutable loci.⁴ An extensive study of the

Ds locus has indicated the reason for this relationship and has produced the information required to interpret the events occurring at mutable loci. It has been concluded that the changed phenotypic expressions of such loci are related to changes in a chromatin element other than that composing the genes themselves, and that mutable loci arise when such chromatin is inserted adjacent to the genes that are showing the variegated expression. The events occurring to this inserted chromatin are reflected in a changed expression of the neighboring genes, or sometimes in a loss of these genes. It is the inserted material that is undergoing the "mutational" events. The Ds locus is composed of this kind of material.

Various types of alterations are observed as the consequence of events occurring at the Ds locus. Some of these alterations resemble the effects produced by x-rays, ultra-violet light, chemicals, etc. They involve chromosome breakage and fusion. The breaks are related, however, to events occurring at this one specific locus in the chromosome—the Ds locus. The Ds designation was given to this locus because the dissociation, now known to be related to dicentric and associated acentric chromatid formation, was recognized before the other events occurring at Ds had been disclosed. Some of the events occurring at Ds , when considered without reference to all the known events, would not by themselves suggest that changed conditions at this locus are associated with a breakage-inducing phenomenon. All of them can be explained, however, by the assumption that one kind of alteration of the inserted chromatin (the chromatin of the Ds locus) takes place, and that the various kinds of changes observed represent consequences of this one altered condition. This condition is assumed to be a stickiness of the

⁴ The annual reports of the author, appearing in the *Yearbooks of the Carnegie Institution of Washington*, 41-48, 1942-1949, contain more detailed summaries of some of the observations that are described in this paper.

materials composing the *Ds* locus, which arises only at precise times in the development of a tissue. The control of the timing of this changed condition will be considered shortly. The reasons for assuming the change to be a stickiness will be obvious from the following list of known events that involve the *Ds* locus. These are: (1) Dicentric chromatid formation with fusion of sister chromatids at the location of *Ds*. This is accompanied by formation of an acentric fragment composed of the two sister segments of this arm, from *Ds* to the end of the arm. (2) Loss of detectable *Ds* activity without visible alteration of the chromosome. In some cases, the loss of *Ds* activity is presumably due to loss of the locus itself. (3) Deletions of chromatin segments of various lengths adjacent to *Ds*, usually with concomitant loss of *Ds* activity but occasionally without loss of this activity. (4) Reciprocal translocation involving chromosome 9 in which one breakage point is at *Ds*. (5) Duplications of segments of chromosome 9, inversion or ring chromosome formations involving chromosome 9 with one break at the *Ds* locus. (6) Transposition of *Ds* activity from one position to another in the chromosomal complement with or without an associated gross chromosomal rearrangement. (7) Changes at the *Ds* locus itself which result in precise changes in the relative frequency of occurrence of the above types of events in future cell and plant generations. This last event, which is of considerable importance, has been termed "change in state" of the *Ds* locus. From a study of the progression of changes in state of *Ds* through cell and plant generations, it appears that the various states may reflect the quantity of the inserted chromatin, the *Ds* loci with larger quantities of this material showing a high frequency of consequences (1), (3), (4), (5) and

(6) above, and those with less of this material showing high frequencies of consequence (2) above.

It is from the transpositions of *Ds* that some of the new mutable loci may arise. The mechanism of transposition has received considerable study. Some cases of transposition of *Ds* are associated with a gross chromosomal rearrangement. In these cases, two chromosome breaks occur to give rise to the rearrangement; one break marks the known position of *Ds* in the chromosome, before the rearrangement occurred, and the second break marks the new position of *Ds* activity. Sister chromatids are affected at each of these two positions of breakage. It has been determined for several of these cases that the appearance of *Ds* activity at the new position most probably arose at the time of origin of the gross chromosomal rearrangement. One case of transposition of *Ds* has been of particular importance because it illustrates how new mutable loci, associated with changes in genic expression, can arise. This transposed *Ds* locus appeared in a single gamete of a plant carrying chromosomes 9 with the dominant *C* allele. This gamete carried a *Ds* locus that had been transposed from a known position in the chromosome 9 to a new position in the same chromosome. The chromosome having *Ds* at this new position was morphologically normal in appearance. This new position of *Ds* corresponded to the known location of *C* (*C*, colored aleurone, dominant to *c*, colorless aleurone). All of the above-enumerated events were now occurring at this new position. Significantly, the appearance of *Ds* activity at this new location was correlated with the disappearance of the normal action of the *C* locus. The resulting phenotype was the same as that produced by the known recessive, *c*. It has been determined from previous studies that a deficiency of the *C* locus

will give rise to a *c* phenotype. That the *c* phenotype in this case was associated with the appearance of *Ds* at the *C* locus, and was not due to a deficiency, was made evident because mutations at this locus from a *c* to a full *C* phenotypic expression occurred. It could be shown that when *C* action reappeared, the *Ds* action concomitantly disappeared from this locus. The restored action of *C* was permanent; no further *Ds*-type events occurred at this *C* locus. In most cases, the event giving a restored *C* action did not result in an altered morphology of chromosome 9. Loss of *Ds* activity without concomitant structural alterations of the chromosome result from event (2) above.

The other enumerated events associated with *Ds* activity were also occurring at this mutable *c* locus. The dicentric chromatid formations were not associated with the appearance of a *C* phenotype, suggesting that the inserted inhibiting material composing *Ds* may be situated proximal to the *C* locus. Several cases of transposition of *Ds* from this location to still another location in the short arm of chromosome 9 were recognized. In each case, a restored *C* action was associated with a disappearance of *Ds* activity at the *C* locus and the appearance of *Ds* activity at the new position. The changes in state of *Ds* at this mutable *c* locus (event (7) above) are particularly significant since it has been determined that a specific change in state of *Ds* is often accompanied by a specific change in the frequency of *c* to *C* mutations.

The origin and behavior of this mutable *c* locus has been interpreted as follows: Insertion of the chromatin composing *Ds* adjacent to the *C* locus is responsible for complete inhibition of the action of *C*. Removal of this foreign chromatin can occur. In many

cases, the mechanism associated with this removal results in restoration of the former genic organization and action. The *Ds* material and its behavior are responsible for the origin and the expression of instability of the mutable *c* locus. The mutation-producing mechanisms involve only *Ds*. No gene mutations occur at the *C* locus; the restoration of its action is due to the removal of the inhibiting *Ds* chromatin. The possible nature of the inserted material will be considered later.

In the cultures having *Ds*, other mutable loci continue to arise. They show types of behavior similar to that described for the mutable *c* locus. This mutable *c* locus (called *c-m1* because it was the first of the mutable *c* loci isolated in these cultures) belongs to the (*a*) group of mutable loci. In some of the progeny of the original self-pollinated cultures, other mutable *c* loci have arisen from previously normal *C* loci. One of these, *c-m2*, shows the type (*c*) expression of variegation, which differs markedly from that shown by *c-m1*. A wide range of quantitative expression, for at least two different reactions associated with aleurone pigment formation, appears as the consequence of various mutations at this locus. The intermediate alleles, full wild-type alleles and some alleles showing even stronger phenotypic expressions than the wild-type from which it arose, are produced by mutations at *c-m2*. The mutations are often expressed as twin sectors, the depth of color in one sector being greater than that in the sister sector. These twin sectors may reflect a single mutation-producing event at the *c-m2* locus that involved both sister chromatids. It has also been determined that chromosome breakage may occur at this locus.

The phenotypic expressions resulting from mutations of *c-m2* and *c-m1* are clearly quite different. That this

difference may be related to differences in the inserted chromatin is suggested by the appearance of a mutable *wx* locus arising from a *Wx* locus in a gamete of a plant carrying *c-m2* (*Wx*, starch of endosperm stains blue with iodine; *wx*, recessive allele, starch stains red with iodine; located in short arm of chromosome 9, proximal to *C*). The type of variegation expressed by this mutable *wx* locus (*wx-m1*) is strikingly similar in all respects to that occurring at *c-m2*. It could not be determined in this case that transposition to the *Wx* locus of the same inhibiting substance that induced *c-m2* had occurred. Such an event is suspected from the known transposition capacities of this material.

In this report, *Ds*, *c-m1*, *c-m2* and *wx-m1* have been used as illustrations of newly arising mutable loci because all of them require an activator and all respond to the same activator. This activator has been designated *Ac*. Extensive studies of *Ac* have shown that it is inherited as a single unit. It shows, however, a very important characteristic not exhibited in studies of the inheritance of the usual genetic factors. This characteristic is the same as that shown by *Ds*. Transposition of *Ac* takes place from one position in the chromosomal complement to another—very often from one chromosome to another. Again, as in *Ds*, changes in state may occur at the *Ac* locus. These changes in state are of two main types: either changes that resemble the known effects produced by different doses of the *Ac* locus from which it was derived, or changes that result in a decidedly altered time of action and dosage response of *Ac*. *Ac* may be detected and its action studied by observing the mutations occurring at the mutable loci requiring its presence for mutability to be expressed. It should be emphasized that when no *Ac* is

present in a nucleus, no mutation-producing events occur at *c-m1*, *c-m2* or *wx-m1*; nor are any chromosome breakage events detected at *Ds*, for no such events occur. As an example of this interaction it may be stated that *c-m1* has been maintained in cultures having no *Ac* locus for several generations, and has given completely colorless aleurone with no evidence of *c* to *C* mutations. Similarly, the various quantitative alleles arising from mutations of *c-m2* or *wx-m1* may be maintained without giving mutations, if *Ac* is removed from the nucleus by appropriate crosses. Thus a series of stable recessive mutations or stable alleles of a mutable locus may be isolated and maintained (if the chromosome complement is normal, see below). When *Ac* is returned to the nucleus, however, instability may again appear.

The dosage action of *Ac* may be studied in the diploid plant or in the triploid endosperm tissue of the kernels. When marked dosage effects are produced by a particular state of *Ac*, they are registered alike in both the plant and the endosperm tissues; the higher the dose of *Ac*, the more delayed is the time of occurrence of mutations at the *Ac*-controlled mutable loci. *Ac* determines, therefore, not only the mutation process at these mutable loci but also the time at which the mutations occur, the different states of *Ac* giving different times of occurrence in 1, 2 or 3 doses. The action of *Ac* on the mutable loci it controls has been described. It is believed that this action produces a stickiness of the inhibiting materials adjacent to the affected loci. With reference to *Ds*, the observed consequences of this stickiness have been enumerated. This physical change probably takes place in the inserted inhibiting materials at all the *Ac*-controlled mutable loci at

the same time in the same cell. This latter conclusion rests on the observation that mutations occur concomitantly at two or more *Ac*-controlled mutable loci when these are present in the same nucleus. The similarity in the type of inheritance and the behavior of *Ds* and *Ac* has been indicated above. Another similarity is that changes in state, loss or transposition of *Ac* occur at the same time that changes take place at the *Ac*-controlled mutable loci. It would appear that the changes in the physical properties of the specific inhibiting chromatin at the mutable loci and at *Ac* itself are of the same nature, and that all are expressions of the primary genetic action of the material composing *Ac*. It is suspected that *Ds* and *Ac* are composed of the same or similar types of material. The possible composition of this material will be considered shortly.

The study of *Ac* and the *Ac*-controlled mutable loci has made it possible to interpret the many patterns of variegation exhibited by mutable loci. The variegated pattern is an expression of the time and frequency of occurrence of visible changes in the phenotype. The frequency of appearance of a visible mutation need not reflect the frequency of the events that occur at a mutable locus, as the study of *c-m1* has clearly revealed. The visible mutations reflect only the frequency of one or several particular consequences of one primary type of event occurring to the inhibiting material adjacent to the affected gene. The changes in state of this inhibiting material that arise as one of the consequences of the primary event, lead to changes in the relative frequency of the consequences of this event when it again occurs in future cell and plant generations. Such changes in state are reflected either in increases or decreases in the relative

frequency of appearance of visible mutations. The study of *Ac* has indicated the nature of the control of the time when the mutations will occur at these mutable loci. The different doses of *Ac* together with the changed states of *Ac* control the time of occurrence of these mutations. The changes in time of occurrence of visible mutations are thus reflections of changes in dosage or changes in state of *Ac*.

The mutable loci that require no activator show the same kinds of expression of variegation as do the activator-requiring mutable loci. It has been shown that the changes occurring at *Ac* are much the same as those occurring at *Ds*. Thus, *Ac* or *Ac*-like loci, could be responsible for the origin of new mutable loci when transposed to a position adjacent to a gene whose inhibited action could be detected by a visible change in phenotype. Dosage action could be exhibited by such autonomous mutable loci, as well as various "changes in state," reflected by changes in the phenotype expression and the time and frequency of occurrence of visible mutations of the affected genes. The study of the behavior of *Ds* in its several states makes it possible to reinterpret the variegation patterns in *Drosophila*, which in some cases appear to be associated with loss of segments of chromosomes and in other cases appear to be associated with changes in the degree of action of the genes involved. It also makes it possible to interpret the reported "position-effect" in *Oenothera*, because the events responsible for the changes in phenotype and the appearance of duplications and deficiencies in this organism appear to be the same or similar to those described for *Ds* in maize.⁵

The possible composition of *Ac* may

⁵ Catcheside, D. G., *J. Genet.* 38:345-352, 1939; *Ibid.* 48:31-42, 1947; *Ibid.* 48:99-110, 1947.

now be considered. Until recently, the investigation was not focused on this problem. It is believed, however, that this material is probably heterochromatin. This statement is based, in part, on the evident homologies in the expression of variegation in maize and *Drosophila*, but is more convincingly suggested by the results of a preliminary experiment focused on the induction of mutations at the a_1 locus in maize when the known *Dt* (Dotted) locus is absent. The action of *Dt* in chromosome 9 on the a_1 locus in chromosome 3 is very much the same as the action of *Ac* on the mutable loci it controls.⁶ The similarities are too great to be dismissed as being due to causally unrelated phenomena. The *Dt* locus activates the a_1 locus; mutations to higher A_1 alleles occur (A_1 , colored aleurone; a_1 , colorless aleurone, recessive to A_1). Without *Dt* in the nucleus, a_1 has been shown to be completely stable. *Dt* is located in the *heterochromatic* knob terminating the short arm of chromosome 9. The suspicion is immediately aroused: Is *Dt* action caused by some modification of the heterochromatic knob in chromosome 9? If so, could this modification be produced anew by subjecting a chromosome 9 to the breakage-fusion-bridge cycle? Would the effective alterations of the knob arise directly because of the induced changes, or would they be produced secondarily by some other induced structural alteration, either within the short arm of chromosome 9 or elsewhere, that would upset, in some way, the normal functioning of the knob substance and thus bring about an alteration in its action? This last question is pertinent because some of the structural alterations in Dros-

phila appear to affect the functioning of the centrically placed heterochromatin. For example, some of the Minutes bring about chromosome elimination and "somatic-crossingover," both of which may well be related to adhesions of specific heterochromatin that occur at certain times in development.⁷ To answer the above questions, plants homozygous for a_1 and having no *Dt* locus (designated *dt* by Rhoades) were crossed by plants similarly constituted with reference to a_1 and *dt* but carrying a rearrangement of the short arm of chromosome 9 that would introduce a chromosome 9 with a newly broken end into many of the primary endosperm nuclei in the given cross.⁸ Breakage-fusion-bridge cycles involving such a chromosome 9 with a newly broken end would occur during the development of the kernels. Some of these broken chromosomes 9 would carry a knob, and this knob could then be subjected to modifications as a consequence of the breakage events. If some of these modifications gave rise to the same conditions that were present at *Dt*, mutations from a_1 to A_1 could appear in some of the kernels resulting from the cross. A large number of crosses of this type were made. The results were positive with respect to inducing mutations of a_1 to A_1 . A small number of the kernels resulting from these crosses showed mutations of a_1 to A_1 . Often, only a single small A_1 spot was present on the kernel. Several of the kernels, however, had a pattern of mutations of a_1 to A_1 that was indistinguishable from that produced by *Dt*. These kernels could not have arisen by contamination, for stocks with the known *Dt* locus had never been obtained and thus no plants with this locus could have been pres-

⁶ Rhoades, M. M., *Genetics* 23:377-397, 1938. *Cold Spring Harbor Symposia Quant. Biol.* 9:138-155, 1941; *Proc. Natl. Acad. Sci.* 31:91-95, 1945.

⁷ Stern, C., *Genetics*, 21:625-730, 1936.

⁸ McClintock, B., *Ibid.* 26:234-282, 1941.

ent in the field. Furthermore, the stock having a_1 and dt , originally obtained from Rhoades, had been grown for several years. A number of sib crosses were made each year and no mutations of a_1 to A_1 were observed in the kernels on these ears.

The facts (1) that Dt is located in the heterochromatic knob of chromosome 9, (2) that the effect it produces can be recreated by subjecting chromosome 9 to the breakage-fusion-bridge cycle, (3) that Ac appeared in stocks that had undergone this cycle, and (4) that Ac and Dt are alike in their respective actions, all point to heterochromatin as the material composing Ac . The burst of new mutable loci which appeared in the self-pollinated progeny of plants that had been subjected to the chromosome type of breakage-fusion-bridge cycle becomes comprehensible if it is considered that the alterations in the quantity or structure of heterochromatic elements during this cycle were primarily responsible for the initial appearance of these mutable loci. This report has shown that, once such loci arise, other mutable loci arise through transposition of the inhibiting chromatin substances to other loci which in turn become mutable.

Why should altered heterochromatin be responsible for initiating such a chain of events? To answer this question, attention must be centered on the action of heterochromatin in the normal nucleus. That it is associated with the exchange of materials between nucleus and cytoplasm has been indicated.⁹ Changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind

and the relative effectiveness of genic action. There can be little question that transpositions of both Ds and Ac occur and that the time of their occurrence in the development of a tissue is under precise control. This control is determined by the number of Ac loci present and their organization and possibly their position in the chromosome complement. Is this transposition of heterochromatin? Is it a reflection of a process that normally occurs in nuclei? Is it responsible for controlling the rates and types of exchange that occur between nucleus and cytoplasm? Is it usually an orderly mechanism, which is related to the control of the processes of differentiation? If so, induced disturbances in quantity and organization of the heterochromatic elements of the chromosome could give rise to a series of alterations reflected both in chromosome structure and behavior and in genic reactions that could markedly alter phenotypic expressions.¹⁰ It is well known that the various knobs and centromeres may coalesce in the resting nuclei. This coalescence is also frequently observed both in the somatic and the meiotic prophases. Are the transpositions and the changes in state of Ac products of this coalescence? This is suspected because of the frequent transpositions of Ac from one chromosome to another.

It may be considered that these speculations with regard to heterochromatin behavior and function have been carried further than the evidence warrants. This may be true; but it cannot be denied that one basic kind of phenomenon appears to underlie the expression of variegation in maize.

¹⁰ This report deals only with the origin and behavior of mutable loci arising in these cultures. A number of other heritable changes are also arising. Many are associated with marked alterations in morphological characters.

In many cases, there can be little question about the similarities in expression of variegation in *Drosophila* and maize. A heterochromatic element has repeatedly been found to be basically associated with the origin and expres-

sion of variegation in *Drosophila*. That a heterochromatic element likewise is responsible for the origin and behavior of variegation in maize has not been proved, although it is indicated, as the analysis of *Dt* has shown.



Some Recent Studies Bearing on the One Gene-One Enzyme Hypothesis

N. H. HOROWITZ and URS LEUPOLD

Reprinted by authors' and publisher's permission from *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 16, 1951, pp. 65-72.

*As Beadle and Tatum pointed out (see p. 166) in their introduction of *Neurospora* to genetics, the concept of the direct one-to-one relationship of genes and enzymes had been suggested and hinted at by many authors prior to themselves. There can be little doubt, however, that the work on the biochemical syntheses of *Neurospora* rapidly became the most solid and believable basis for the one gene-one enzyme hypothesis. Careful reading of the Beadle and Tatum paper, particularly in the experimental procedures, will show it was to be anticipated that these tests would result in evidence of a one gene-one enzyme relationship, because more complicated relationships would not be demonstrable by these procedures. This difficulty was disturbing even to the supporters of the hypothesis. In the following paper, Horowitz and Leupold describe a series of experiments which eliminate the essentially negative aspect of previous studies, and which give conclusive and positive evidence as to the frequency of occurrence of the unitary relationships of genes and enzymes. It is delightful to note the way their evidence supplements earlier knowledge, and at the same time implements further investigation. Thus, indeed, does a science grow.*

THE ASSUMPTION THAT A GIVEN GENE IS INVOLVED, IN A PRIMARY WAY, IN THE

PRODUCTION OF BUT A SINGLE ENZYME HAS BEEN IMPLICIT IN MOST SPECULATIONS ON

the nature of gene action since Cuénot's time. As a result of the investigations of the last ten years stemming from the discovery of nutritional mutants in *Neurospora* by Beadle and Tatum (1941), one is now in a position to scrutinize this supposition more closely than was previously possible. Specifically, we are in a better position to trace the consequences of the hypothesis and of its various alternatives, and to appraise the evidence which may have a bearing on it. In this paper we propose to examine some of the evidence, deriving from studies on *Neurospora*, and *E. coli*,¹ which relates to this problem.

Before considering the experimental findings, it may be useful to define more explicitly the meaning of the one gene-one enzyme hypothesis. The concept is that of a gene whose sole activity aside from self-duplication is that of functioning in the synthesis of a particular enzyme or enzyme precursor. It is not thereby implied that genes at other loci may not also function directly in the formation of the enzyme. This is a completely independent problem with which we are not concerned here, and regarding which there is little evidence in *Neurospora* one way or the other; all that can be said with assurance is that if two or more genes do, in fact, cooperate in the production of a given enzyme, then their respective contributions must be different. Nor does the one gene-one enzyme hypothesis imply that the final phenotypic ex-

pression of a mutation is necessarily restricted to a particular structure or function of the organism. The ultimate effect of a mutation is the result of an enormous magnification of the initial gene change, brought about through a system of reactions which, originating at the gene, rapidly branches out in various directions and coalesces with similar networks deriving from other loci to form a reticulum of as yet indeterminate extent and complexity. It is impossible to decide from the end-effects alone whether the gene has one or many primary functions, since on either assumption a complex pattern of effects is expected in most cases. In the biochemical mutants of *Neurospora* and other micro-organisms, the end effects would, if they could be analysed, undoubtedly prove to be exceedingly numerous. A mutation which induces a deficiency of an amino acid, for example, must secondarily affect the synthesis of virtually every protein of the cell, and an exhaustive enumeration of the end effects might well include every structure and function of the organism.

It turns out, however, that it is possible in such a case to prevent the secondary damage and the consequent death of the mutant by supplying the lacking amino acid. When given a sufficient quantity of the amino acid the mutant becomes normal in growth rate, morphology, and fertility. It is difficult to escape the conclusion that the sole function of the gene in this case is to play some essential role in the synthesis of the amino acid. When biochemical analysis of the mutant is carried farther, it is discovered that the field of action of the gene is even more circumscribed than might have been supposed: it is restricted to sensibly a single chemical step of the synthesis. Apparently a single reaction is abolished in the mutant, while all

¹ The studies on *E. coli* reported in this paper were supported by a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council; by a grant from the Rockefeller Foundation; and by a contract between the Office of Naval Research, Department of the Navy and the California Institute of Technology (NR 164010).

others proceed normally. It is inferred that the role of the gene is to function in the synthesis of the enzyme which catalyses this reaction.

It has not yet been possible to analyse all, or even the majority, of the known *Neurospora* mutants in the detail we have just outlined, while in a few cases the analysis has been carried still farther by showing that the mutants are in fact lacking in particular enzymes (Mitchell and Lein, 1948; Fincham, in press). Out of approximately 500 nutritional mutants which are, or have been, in the Pasadena collection, 84 per cent require single, known chemical substances for growth. The remaining 16 per cent have not responded to any of the individual substances tested, but do grow on complex media. It is very likely that many of the strains in this unanalysed group require individual compounds which have not been tested, others may have multiple requirements resulting from multiple mutations, while some may have multiple requirements resulting from the mutation of multi-functional genes.

The one gene-one enzyme hypothesis has been suggested as the simplest interpretation of the large class of mutants whose growth requirement is known to be satisfied by a single growth factor. Are there any grounds for suspecting that these mutants may not, in spite of appearances, represent mutations of unifunctional genes?

One basis for criticism of the one gene-one enzyme interpretation is the difficulty of excluding in every instance the alternative hypothesis that the given gene controls not one, but several sequential steps in the affected pathway. This interesting idea appears rather improbable, however, in view of the cumulative evidence from series of mutants which shows that each gene can be assigned to a particular step in

a sequence of reactions; and it is virtually excluded in those cases where it has been possible, by enzyme studies, to define the reaction precisely.

A second ground for suspicion of the one gene-one enzyme interpretation rests on the fact that closer study of the mutants shows that they are not in all cases restored to a fully normal phenotype when supplied with the required growth substance. While all of the lethal consequences of the mutation are avoided, a residue of non-lethal effects may remain. In some cases, these residual effects are readily accounted for—partial sterility, for example, when the mutant carries a chromosomal rearrangement. Others are not so easily understood. One of the commonest residual effects is a sensitivity toward certain natural substances—frequently amino acids. The first reported instance of this phenomenon was that described by Doermann (1944), who found that growth of all of the then known lysine-requiring mutants—a series involving at least three loci—is competitively inhibited by L-arginine in the medium. The growth of wild type *Neurospora* is not affected by arginine. Many similar cases are now known. A significant feature of this phenomenon is that the inhibition may, as in the case cited, extend to a whole class of genetically different, but biochemically related mutants, indicating that the effect is not locus-specific but is inherent in the mechanism of utilization of the exogenously provided growth substance. A third residual effect which has been encountered is sterility in crosses in which both parents carry the same mutant allele. One interpretation is that in these cases the gene performs a specific function, possibly independent of its vegetative biochemical function, in connection with zygote formation or maturation. Some recent prelimi-

nary results which have been obtained in our laboratory by Mr. Henry Gershowitz, working with certain methionine-requiring strains, indicate, however, that the sterility can be overcome by supplementing the medium with a large quantity of the amino acid—at least twice as much as is required to produce optimal growth of vegetative cultures. This suggests that the sterility may result from a high metabolic requirement for the growth factor during the sexual process, or to a lowered permeability to it. A fourth, and relatively rare, residual effect is failure of the mutant to attain a normal growth rate. This also can characterize an entire class of mutants, as in a certain group of strains of the cysteine-methionine series now under investigation in our laboratory.

Everything considered, it is perhaps surprising that residual effects are not observed more frequently, since in no event is it possible, even in theory, to avoid all of the consequences of a mutation by supplying the deficient metabolite. The block in the synthetic pathway still remains, and it can have an influence quite apart from the effects of the nutritional deficiency. It has been shown in numerous instances that metabolic intermediates may accumulate behind the block, sometimes in spectacular quantities (for review, see Horowitz, 1950). It would be surprising if the presence of abnormal concentrations of metabolic intermediates in the cells did not at times produce deleterious side-effects. Actually, evidence has been obtained both in *Neurospora* (Bonner, 1946a) and in *E. coli* (Davis, 1950; Umbarger and Mueller, 1951) that accumulated intermediates may exert a lethal action by interfering with reactions in other metabolic pathways. This leads one to

suspect that the so-called residual effects are to a large extent the irreparable side-effects of the primary block. (For further discussion, see Emerson, 1950.)

THE SELECTION PROBLEM

At the Cold Spring Harbor Symposium of 1946, Delbrück raised a question as to whether incompatibilities with the one gene-one enzyme hypothesis could be detected even if they occurred (see Discussion following paper by Bonner, 1946b). Delbrück's argument was based on the recognized fact that not all of the mutations which are produced can be detected by the methods usually employed for this purpose. Principally three classes of biochemical mutants are not recoverable: (1) those requiring a substance which is absent from the so-called "complete" medium used for recovering nutritional mutants, (2) those requiring a substance which is unable to diffuse into the cell, and (3) those requiring a substance which, though present and diffusible, is not utilized because of the inclusion in the medium of an inhibitor of the mutant in question. We shall refer to mutants which, for the above, or for any other reasons, are incapable of growing on complete medium as mutants which have lost an *indispensable function*. The point of Delbrück's argument was that if any gene has more than one primary function, it is likely that at least one of these is an indispensable function; in which case mutation of the gene would not be detected.

Now the validity of this argument depends on the relative frequency of indispensable functions. If this frequency is very high, then the probability of recovering a mutation of a gene with several primary functions will be very low. Thus, if 90 per cent

of gene functions are indispensable, and if dispensable and indispensable functions are randomly distributed among the genes, then the probability of detecting a mutation in a gene with two primary functions is only one per cent. On the other hand, if the frequency of indispensable functions is low, then the chance of detecting multifunctional genes will be much better. The determination of the proportion of indispensable functions is thus critical for the one gene-one enzyme concept. The question is how this quantity is to be determined. It would seem almost by definition to be unknowable, in which case the one gene-one enzyme idea must be banished to the purgatory of untestable hypotheses, along with the proposition that a blue unicorn lives on the other side of the moon.

THE FREQUENCY OF INDISPENSABLE FUNCTIONS IN *NEUROSPORA*

What is needed is a method for detecting mutations which result in loss of an indispensable function and for comparing their frequency to that of mutations which cause loss of a dispensable function. It occurred to one of us (Horowitz, 1948, 1950) that the so-called "temperature mutants" of *Neurospora* might form the basis of such a method. Temperature mutants are a class in which the mutant phenotype is fully expressed only in a particular temperature range. Generally, such mutants exhibit a growth factor requirement when cultured at 35°, but grow in its absence at 25°; in a few cases this relationship is reversed —i.e., the growth factor is required at the lower, but not at the higher, temperature. In three instances it has been found that particular temperature mutations behave as alleles of mutations of the usual, temperature-independent

sort, and it seems not unlikely that this will be found to be generally true.

The usefulness of these mutants for the present problem is based on the expectation that the mutant will be recoverable in the temperature range within which it has no growth factor requirement, regardless of whether a dispensable or an indispensable function has been lost. This expectation is borne out by the fact that a group of temperature mutants which fails to grow on complete medium at the mutant temperature is, in fact, known. Of the 26 temperature mutants known in *Neurospora*, 12 are of this type, while 14 grow on complete medium in the temperature range within which they have a requirement. In other words, roughly one-half of these mutants has lost an indispensable function.

In using the temperature mutants as a sampling device it is assumed that genes controlling indispensable functions are just as likely to yield temperature alleles as those controlling dispensable functions. This assumption is supported by two considerations. In the first place, the two classes of functions are in no sense natural categories, but depend largely on the composition of the particular complete medium which is employed. There is thus no reason to assume that the genes governing these functions differ from one another in any fundamental way. In the second place, among the temperature mutants whose specific requirement is known there is no indication that any one kind of nutritional requirement is favored over others. Mutation to temperature alleles appears to occur at random among genes controlling known biochemical syntheses (Horowitz, 1950).

With the information that the frequency of indispensable gene functions constitutes approximately 50

per cent of the total, it becomes possible to estimate the intensity of the selection which operates against the detection of multifunctional genes. With a random distribution of functions, one-half of genes with a single function will be detectable by the usual methods, one-fourth of bifunctional genes, and, in general, $(\frac{1}{2})^n$ of n -functional genes. The original minimal estimate of 84 per cent of unifunctional genes, based on the observation that this fraction of the mutants responds to single growth substances can now be corrected. A sufficiently close approximation is given by neglecting genes with more than two functions, and we obtain 73 per cent as the corrected frequency of unifunctional genes:

$$\text{Observed frequency} =$$

$$\frac{84}{84 + 16} = 0.84$$

$$\text{Corrected frequency} =$$

$$\frac{84 \times 2}{84 \times 2 + 16 \times 4} = 0.73$$

The exact value is given by the first term of a Poisson distribution, and is equal to 0.71 (see Appendix).

This value is so high, that in spite of the uncertainties in its determination it may be regarded as strongly supporting the conclusion that at least the majority of genes controlling biosynthetic reactions in *Neurospora* are unifunctional. There are several obvious sources of uncertainty in the calculations. First, they should be based on the number of genetically different mutations, rather than on the total number of occurrences; this cannot be done at the present time. Second, the assumption was made that all of the unanalysed mutants, 16 per cent of the total, represent multi-functional genes; this is almost certainly incorrect and biases the calculations against the one gene-one enzyme theory. Finally, the number of temperature mutants is

too small to give an accurate estimate of the frequency of indispensable functions. It is to the last point that we now turn.

THE FREQUENCY OF INDISPENSABLE FUNCTIONS IN *E. COLI*

It was clearly desirable to obtain a more reliable estimate of the frequency of indispensable functions, but to even double the existing number of temperature mutants in *Neurospora* would be a formidable operation. We therefore turned to *E. coli* K-12, with the expectation of recovering large numbers of temperature mutants by a modified penicillin technique (Davis, 1948; Lederberg and Zinder, 1948). Providentially, this method proved to be unsuited to our purpose: although temperature-independent mutants were obtained, the yield of temperature mutants was zero. This was a fortunate circumstance, since it forced us to adopt a more direct method, one which introduces fewer uncontrolled selective variables into the experiment than would the penicillin technique. The method is simply that of plating out U.V.-treated cells on minimal medium and incubating them for 48 hours at 40°. The plates are transferred to 25° for an additional 5 days, and the colonies which come up during this second period—so-called secondary colonies—are picked off and tested. This procedure was made feasible by a visual method devised by Dr. Leupold which makes it easier to detect a few secondary colonies on a plate containing hundreds of primary colonies. Altogether 161 temperature mutants were obtained by this method. Of these, only 37, or 23 per cent, were unable to grow on the *Neurospora* complete medium at 40° and therefore represent losses of indispensable functions. The statistics are shown in Table 1.

TABLE 1
Statistics of *E. coli* Study

No. of irradiated cells	1.7×10^9 (approx.)
No. of surviving cells	2.4×10^6 (approx.)
Secondary colonies	
isolated	2157
No. of temperature mutants	161
Type D40	124
Type I40	37

The remaining 124 mutants, those which grow on complete medium at 40°, were tested by the auxanographic method to determine their growth requirements. Seventy-nine per cent of these mutants were classifiable in this way. A variety of requirements was found (Table 2), indicating again that

TABLE 2
Syntheses Known To Be Affected
in *E. coli* Temperature Mutants

Amino Acids	Vitamins
Methionine	Biotin
Cystine	Thiamin
Arginine	Pyridoxin
Lysine	Nicotinamide
Histidine	Pantothenic acid
Leucine	
Isoleucine	
Valine	Nucleic Acid Constituents
Threonine	
Aminobutyrate	
Tyrosine	
Glycine	

temperature mutation is random with respect to the classes of syntheses which can be affected. A number of substances are conspicuous by their absence from this list, notably tryptophane and *p*-aminobenzoic acid. It has not been excluded, however, that requirements for these substances are present among the mutants which were not classifiable in the auxanographic test.

Several other points of interest in connection with the *E. coli* study should be mentioned. These concern the selective forces operating in densely populated versus lightly populated Petri plates. It is obvious that in a method like this, in which there may be many hundred of colonies per (15 cm.) plate, we are not actually isolating the mutants in a minimal medium, but in a minimal medium plus or minus whatever the hundreds of wild type colonies add to or subtract from it. This is quite clearly shown in the relative yield of secondary colonies and temperature mutants per number of survivors. In our experiments the total number of colonies (i.e., survivors) per plate varied from 100 to 1,700. In sparsely populated plates the relative yield of both secondary colonies and temperature mutants was much higher than in densely populated plates. In Figure 1 the numbers of secondary colonies and temperature mutants per million survivors are plotted against the total number of colonies per plate. At the lowest densities, with populations of the order of 100 colonies per plate, yields of 3,400 and 6,500 secondary colonies per million survivors have been recorded. At higher densities, the yield decreases systematically and rapidly, reaching values between 200 and 800 secondary colonies per million survivors at population densities of 1,200 to 1,700 colonies per plate. The yield of temperature mutants per million survivors is roughly one-tenth that of all secondary colonies, and it exhibits the same systematic trend. The difference between these two curves expresses the fact that approximately 90 per cent of the secondary colonies are wild types which, for one reason or another, started to grow late; partially blocked, slowly growing biochemical mutants; and completely blocked bio-

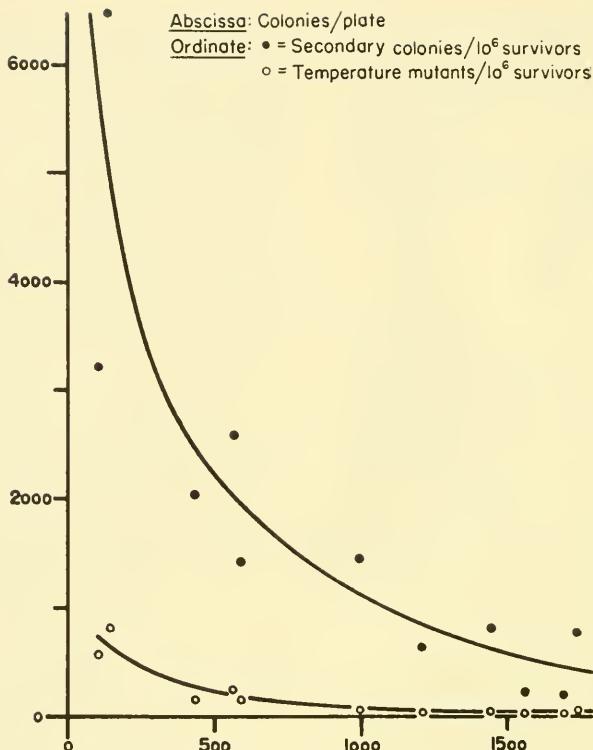


Fig. 1. Frequency of secondary colonies (solid circles) and of temperature mutants (open circles) per 10^6 survivors as functions of population density.

chemical mutants which have been fed "syntrophically" by the wild types. Figure 2 shows that the composition of the population of secondary colonies is also influenced by the population density. It is seen that the yield of temperature mutants per hundred secondary colonies decreases with increasing densities, indicating that the yield of temperature mutants decreases even more rapidly than does the yield of secondary colonies in general.

At least three selective forces are at work in these populations: (1) competition for food, (2) probably more important, mutual inhibition by-products of metabolism, and (3) superimposed on these but acting in the opposite direction, cross-feeding, or syntrophism, the mutual exchange of

essential growth factors. The first two mechanisms are probably mainly responsible for the rapid decrease in the yield of secondary colonies with increasing plate densities. Their intensity is evidently quite remarkable. They are unspecific forces, however, and cannot be expected to influence systematically the relative frequencies of the two types of temperature mutants which we set out to find. The third influence, cross-feeding, is much more dangerous in this respect, although its direction and intensity are difficult to predict. The effect of cross-feeding on the selective isolation of the two kinds of temperature mutants depends on both the quantity and quality of the output of growth factors by wild type *E. coli*. For example, let us assume that

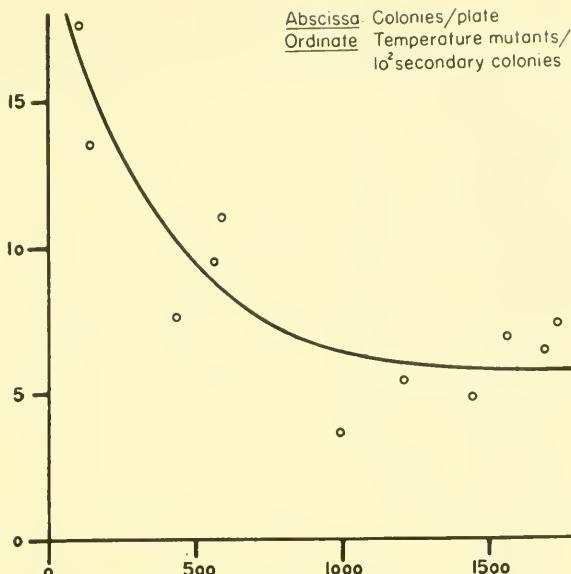


Fig. 2. Frequency of temperature mutants per hundred secondary colonies as a function of population density.

the growth factor excretion by the wild type is qualitatively similar to the composition of complete medium. Large amounts of such enrichment would tend to support the growth of temperature mutants which had lost a dispensable function, up to the point of visible colony formation, and would thus remove them from the isolation procedure. On the other hand, smaller quantities of the same enrichment might give these same mutants an advantage in the struggle for survival during the first 48 hours at the mutant temperature and during the second period at the lower temperature when they begin to grow against the heavy competition of established wild type colonies. The actual situation would be far more complex, in that the sign and magnitude of the selection would differ from mutant to mutant.

In spite of the indisputable occurrence of cross-feeding on the plates, however, it can be said that no systematic selection favoring either of the

two classes of temperature mutants is deducible from our data. In Figure 3 is plotted the fraction of temperature mutants which were found to belong to the indispensable class, together with the ranges within which the values would be expected to fall in 95 per cent of trials, against the population density. With the exception of one experiment, the data are consistent with the assumption that these are random samples drawn from a homogeneous population of temperature mutants in which the frequency of mutants of the indispensable class is between 20 and 25 per cent. In short, with the exception of a single experiment in the middle range of population densities, there is no indication that population density influences the relative frequencies of the two classes of temperature mutants: the various selective forces appear to affect both classes equally.

We may summarize the *E. coli* results, then, by saying that the fre-

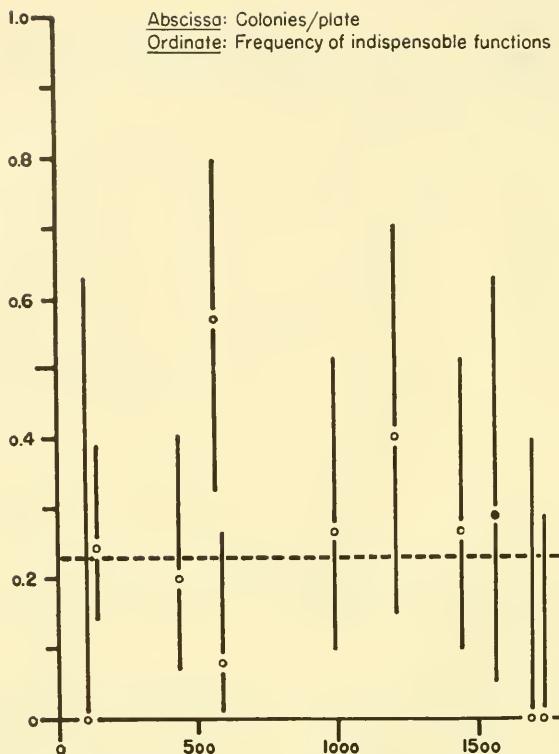


Fig. 3. Frequency of temperature mutants of the indispensable class as a function of population density. Vertical lines show the range within which the experimental points would be expected to fall in 95 per cent of similar experiments. The broken line shows the weighted mean of the distribution.

quency of indispensable functions, as revealed by the temperature mutant method, is even lower than was indicated by the less extensive series of temperature mutants in *Neurospora*. Actually, the two results—46 per cent of indispensable functions in *Neurospora*, compared to 23 per cent in *E. coli*—are not very different, considering the great differences in the respective organisms. The intensity of the selection which opposes the recovery of multifunctional genes in the usual screening procedures is thus of a rather low order and is incapable of accounting for the fact that the genes detected by these procedures appear to be preponderantly of the unifunctional type.

CONCLUSION

In concluding this paper, we should like to make some brief observations on the significance of the low frequency of indispensable functions and the one gene-one enzyme hypothesis.

Our results indicate that the effect of most lethal mutations in *Neurospora* and *E. coli* is to block the synthesis of metabolites which are replaceable by nutritional means; that is to say, of low molecular weight substances such as might be expected to diffuse into the cell and of which the complete medium is chiefly, if not exclusively, composed. This situation appears to contrast markedly with that encountered

in *Drosophila*, where lethal mutations, as well as visibles, result in irreplaceable losses, frequently organ-specific in character. In some measure this may reflect fundamental biological differences between *Drosophila* and *Neurospora* or *E. coli*, but also, and to an indeterminate extent, it reflects the differences in methodological approach to these organisms. It would not be surprising to find that non-diffusible products play a more important role in the development of a highly differentiated organism like *Drosophila* than in relatively undifferentiated ones like *Neurospora* and *E. coli*. But on the other hand, it must be recognized that the methods of *Drosophila* culture are such as virtually to exclude the possibility of detecting nutritional mutants even if they occur.

The results we have obtained from the microorganisms suggest that in the great majority of cases the metabolic function of the gene is to produce an enzyme which catalyzes the formation of a low molecular weight product. If there exists a large class of enzymes whose function is other than this, we must conclude either that they are not individually essential to survival, or else that they are independent of the genes for their production. There is a strong suggestion in this result that the mechanism of biosynthesis of large molecules, especially proteins, is not an enzymatic mechanism in the usual sense. That is to say, the protein molecule may not be built, cathedral-like, by a process of accretion; but rather may be made directly from the amino acids or their simple derivatives by a single catalyst.

It is interesting to note that such a mechanism provides a simple explanation of another essential feature of our findings; namely, the mutual independence of the pathways of synthesis of different enzymes. On the hypothesis

of growth by accretion of peptide fragments it seems unlikely that a one gene-one enzyme relationship could be found, even if it existed, since loss or modification of any peptide fragment would be expected to result in loss or modification of a particular group of enzymes; namely, those which contain this fragment in their structures. Unless the fragment could be introduced into the cells, the result would be the frequent occurrence of multiple unrelated biochemical deficiencies among the mutants. This has not been found. We find considerable appeal in the notion that the proposed mechanism of enzyme synthesis may underlie the results we have obtained.

SUMMARY

The one gene-one enzyme hypothesis is supported by the following evidence:

The great majority, at least 84 per cent, of the known nutritional mutants of *Neurospora* require single chemical substances as essential growth factors. Provision of the essential factor results in a normal phenotype in most cases; where a fully normal phenotype is not attained, the evidence indicates that this is to be accounted for on the basis of secondary effects unrelated to the mode of gene action.

Biochemical analysis of the mutants has indicated in many instances that the effect of the mutation is to block sensibly a single step in the pathway of synthesis of the growth factor. In a few cases it has been possible to show that the mutants are lacking in the specific enzyme involved.

The question of whether the known biochemical mutants are a highly selected sample from which multifunctional mutants are excluded by virtue of the screening procedure employed has been attacked by the temperature mutant method. Evidence has been

presented which indicates that this method makes possible the recovery of mutants without regard to the nature of the induced biochemical deficiency or the composition of the (complete) medium.

Application of this method has shown that the proportion of biochemical mutants not recoverable by the usual screening tests may be less than 50 per cent in *Neurospora* and less than 25 per cent in *E. coli*. It is calculated that this rate of loss does not produce a sufficiently intense selection of unifunctional mutations to account for the high frequency of such mutations actually found.

Finally, it is suggested that the results can be simply accounted for on the hypothesis that the synthesis of a protein molecule is accomplished by a single catalyst working directly on the constituent amino acids or their simple derivatives.

APPENDIX

The corrected value of the frequency of unifunctional genes can be computed from a Poisson distribution as follows:

Assume that each gene has one function to begin with and that there is in addition a number of functions, m , randomly distributed among the genes. The fraction i of all gene functions is indispensable. Letting

$$n = \text{the number of genes}$$

Then $n + m = \text{the number of gene functions}$,

$$\frac{m}{n} = c = \text{the mean number of additional functions per gene,}$$

And ic = the mean number of indispensable additional functions per gene.

The fraction of genes with no additional functions—i.e., unifunctional—is

then given by e^{-c} , the first term of a Poisson distribution. Of these, the fraction $(1-i)$ are recoverable. The fraction of recoverable unifunctional genes is therefore

$$P_a = (1-i)e^{-c}$$

Similarly, the fraction of all genes, unifunctional and multifunctional, which can be recovered is

$$P_{a+b} = (1-i)e^{-ic}$$

The frequency of unifunctional genes among those recovered is therefore

$$P_a/P_{a+b} = e^{-c(1-i)}$$

Equating this expression to the observed value, 0.84, and substituting 0.5 for i , one finds $c = 0.34$. The corrected frequency of unifunctional genes is then $e^{-0.34} = 0.71$.

REFERENCES

- Beadle, G. W. and Tatum, E. L. 1941 Genetic control of biochemical reactions in *Neurospora*. *Proc. Nat. Acad. Sci. Wash.* 27:499-506.
- Bonner, D., 1946a Further studies of mutant strains of *Neurospora* requiring isoleucine and valine. *J. Biol. Chem.* 166:545-554.
- 1946b Biochemical mutations in *Neurospora*. *Cold Spr. Harbor Symposium Quant. Biol.* 11:14-24.
- Davis, B. D. 1948 Isolation of biochemically deficient mutants of bacteria by penicillin. *J. Amer. Chem. Soc.* 70:4267.
- 1950 Studies on nutritionally deficient bacterial mutants isolated by means of penicillin. *Experientia* 6:41-50.
- Doermann, A. H. 1944 A lysineless mutant of *Neurospora* and its inhibition by arginine. *Arch. Biochem.* 5:373-383.
- Emerson, S. 1950 Competitive reactions and antagonisms in the biosynthesis of amino acids by *Neurospora*. *Cold Spr. Harbor Symposium Quant. Biol.* 14:40-48.
- Fincham, J. R. S., *J. Gen. Microbiol.* in press.

- Horowitz, N. H. 1948 The one gene-one enzyme hypothesis. *Genetics* 33:612-613.
- 1950 Biochemical genetics of *Neurospora*. *Advances in Genetics* 3:33-71.
- Lederberg, J. and Zinder, N. 1948 Concentration of biochemical mutants of bacteria with penicillin. *J. Amer. Chem. Soc.* 70: 4267.
- Mitchell, H. K. and Lein, J. 1948 A *Neurospora* mutant deficient in the enzymatic synthesis of tryptophan. *J. Biol. Chem.* 175:481-482.
- Umbarger, H. E. and Mueller, J. H. 1951 Isoleucine and valine metabolism of *Escherichia coli*. I. Growth studies on amino acid-deficient mutants. *J. Biol. Chem.* 189: 277-285.



Genetic Exchange in *Salmonella*

NORTON D. ZINDER and JOSHUA LEDERBERG

Reprinted by authors' and publisher's permission from *Journal of Bacteriology*, vol. 64, 1952, pp. 679-699.

The striking discovery of sex in bacteria (p. 192) was followed a few years later by this paper, which demonstrates an even more unusual phenomenon. Evidence is presented to show that hereditary materials from one strain of bacterium can be carried to a second strain by a completely different organism—a virus. The materials so carried are then transmitted by the second strain in a normal hereditary sequence, through many generations. The relationship between this phenomenon and the occurrence of uncontrolled and presently uncontrollable outbursts of genetic activity in higher organisms is of great significance in medical research, as evidenced by the recognition of Dr. Lederberg's work on this and sexual recombination in bacteria by the Nobel Prize Committee in 1958.

GENETIC INVESTIGATIONS WITH MANY different bacteria have revealed parallelisms and some contrasts with the biology of higher forms. The successful application of selective enrichment techniques to the study of gene recombination in *Escherichia coli* (Tatum and Lederberg, 1947; Lederberg *et al.*, 1951) suggested that a similar approach should be applied to other

bacteria. This paper¹ presents the re-

¹ Department of Genetics, paper no. 479. This investigation was supported by research grants (E72) from the National Microbiological Institute of the National Institutes of Health, Public Health Service, from the Rockefeller Foundation and from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. This work has been submitted by the senior author to the

sults of such experiments with *Salmonella typhimurium* and other *Salmonella* serotypes. The mechanism of genetic exchange found in these experiments differs from sexual recombination in *E. coli* in many respects so as to warrant a new descriptive term, transduction.

MATERIALS AND METHODS

Most of the strains of *S. typhimurium* were provided by Lilleengen (1948) as representative of his 21 "phage types", LT-1 through LT-22. Most if not all strains of *S. typhimurium* are lysogenic (Boyd, 1950), and these have provided 12 lines of bacteriophage. Other cultures were obtained from F. Kauffmann, E. K. Borman, and P. R. Edwards. All cultures were maintained on nutrient agar slants.

Specific growth factor dependent mutants (auxotrophs) were obtained from ultraviolet irradiated cell suspensions subjected to the penicillin method for selective isolation (Davis, 1950a; Lederberg and Zinder, 1948). Similar mutants have been obtained in *Salmonella* by Plough *et al.* (1951) and Bacon *et al.* (1951). Other methods for the isolation and characterization of auxotrophic and fermentation mutants have been documented elsewhere (Lederberg, 1950; Lederberg and Lederberg, 1952). Streptomycin resistant mutants were selected by plating dense, unirradiated cell suspensions into agar containing 500 mg per L of dihydrostreptomycin.

"Complete" indicator medium (EMB) was made up from the same formula as for *E. coli* (Lederberg, 1950). The defined eosin methylene blue medium ("EML agar") contained

in g per L: sodium lactate, 2.5; $(\text{NH}_4)_2\text{SO}_4$, 5; NaCl, 1; MgSO_4 , 1; K_2HPO_4 , 2; methylene blue hydrochloride, 0.05; eosin Y, 0.3; and agar, 15. Difco products, penassay broth, and nutrient agar, were employed as "complete" media.

Unless otherwise stated, all cultures were incubated at 37°C, and plates were scored after 24 and 48 hours.

EXPERIMENTAL RESULTS

Direct crosses: platings of mixed cultures. In *E. coli*, recombinants were detected selectively by plating various auxotrophs together on minimal agar. Both parents are suppressed on this medium and, barring various experimental errors, colony formation is confined to prototrophic recombinant cells. Such errors can be detected by appropriate controls but are best mitigated by the use of double nutritional mutants (diauxotrophs). These are obtained by the iterated isolation of mutants in previously established auxotroph lines.

One of Lilleengen's strains was refractory to our techniques of mutant isolation. Two-step mutants with mutually complementary nutritional requirements were prepared from each of the remaining twenty types. Of the two hundred possible pairwise combinations, including "selfed" crosses, one hundred were tested. Each combination was studied by mixing and plating 10^9 washed cells of the two parents on a minimal agar plate. Fifteen mixture plates and five control plates for each parent by itself were inoculated in each test. Fifteen combinations yielded prototrophs in contrast to barren controls. Strain LA-22 was the most "fertile", especially with LA-2 (see table 1). This cross yielded about one prototroph per hundred thousand parental cells plated. Crosses in which LA-22 was not involved gave

TABLE I

Mutant strains and symbols used

Number	Mutations	Pertinent Symbol
LT-2	Type 2 prototrophic	Prot
SW-272	Methionineless, auxotrophic	Aux
SW-414 (LA-2)	SW-272 histidineless	
LT-22	Type 22 parent	
SW-240	Phenylalanine and tyrosineless	
SW-279	SW-240 tryptophanless	
SW-307	SW-279 galactose-negative	Gal-
SW-351	SW-307 xylose-negative	Xyl-
SW-435	LA-22 SW-351 streptomycin-resistant	Sr
SW-479	SW-435 mannitol-negative	Mtl-
SW-443	SW-435 maltose-negative	Mal-
LT-7	Type 7 parent	
SW-184	Prolineless	
SW-188	Methionineless	
SW-191	Leucineless	
SW-481	SW-184 galactose-negative	
SW-492	SW-188 galactose-negative	
SW-503	SW-191 galactose-negative	
SW-514	LT-7 streptomycin-resistant	
SW-515	SW-503 streptomycin-resistant	

prototrophs so infrequently and sporadically as to be of doubtful significance. It has since become evident that LA-22 is genetically a single, stable mutant although it was derived in two steps and has a complex nutrition.

LT-22 is lysogenic for a virus (hereafter referred to as PLT-22) active on LT-2. This virus is capable of inducing lysogenicity in LT-2. Among the lysogenic derivatives of LA-2 three different interaction groups were found: the majority no longer interacted with LA-22 to give prototrophs; a few interacted with impaired efficiency; still fewer were not affected in this respect. These experiments indicated that genetic exchanges did occur and that latent bacteriophage played some role in the interaction.

Indirect crosses: platings of cells and filtrates. To test the possible role of filtrable factors in this interaction, a U-tube with an "ultra-fine" sintered

pyrex filter partition was prepared according to Davis's (1950b) design. By alternating suction between the arms of the tube, two intact populations of growing bacteria could be made to share the same medium. The integrity of the filter was verified in control experiments by leaving one compartment uninoculated. Then 10^8 cells of each parent were inoculated into twenty ml of broth and placed in either arm of the tube. Ten ml were flushed from side to side every twenty minutes for four hours while the culture grew to saturation. The two populations were washed and plated upon minimal medium. Prototrophs appeared in the platings of LA-22 but not of LA-2. Sterile filtrates of LA-2 broth cultures did not elicit prototrophs from LA-22. However, filtrates from mixed cultures of LA-2 and LA-22 elicited about one prototroph per million LA-22 cells. Thus LA-2

produced a filtrable agent (*FA*), under stimulation from LA-22, that could elicit prototrophs from LA-22. Filtrates of LA-22 cultures, containing substantial amounts of phage (PLT-22) active on LA-2, also stimulated *FA* production from LA-2. The role of this phage will be discussed later.

To help the further exposition of our experiments, we shall use the term transduction for genetically unilateral transfer in contrast to the union of equivalent elements in fertilization. The working hypothesis that *Salmonella FA* is an agent of genetic transduction provides a useful frame of reference for our discussion.

Assay of FA. Stock *FA* was prepared by growing LA-22 and LA-2 in mixed culture in broth. After 48 hours, the cells were sedimented and the supernatant passed through a sintered pyrex filter. The sterility of a filtrate was verified by inoculating samples into broth at the time of preparation and by platings in agar as controls for particular experiments. This precaution was taken although complete sterility is not critical to most experiments since more than a million cells of LA-2 per plate are needed to interact with LA-22 to give prototrophs in the "direct crosses". These preparations have been stored in the refrigerator for several months without loss of activity.

A standard procedure for assay of *FA* was developed for further work. LA-22 was grown on nutrient agar plates and harvested in dense saline suspension. The viable count was obtained by plating suitable dilutions on nutrient agar. Various dilutions of cells were plated with a constant volume of an *FA* preparation on minimal agar. Prototrophs appeared at 24 hours and were counted after 48 hours. Figure 1 shows that a constant response was found with about 10^9 to 10^{10} cells per

plate. The decline at high cell densities was probably due to overcrowding and inhibition of colony formation, and at lower densities to physical separation of cells and agent or to the saturation of susceptible cells.

10^9 cells of LA-22 were plated with serial dilutions of *FA*. Over a considerable range a linear relationship was found between the yield of prototrophs and amount of *FA* (figure 1). The effect of higher concentrations of *FA* will be discussed in a later section.

A unit of *FA* may be defined as the content of a filtrate that will elicit a single prototroph from an optimum concentration of LA-22 cells. Filtrates from mixed cell preparations usually contain about 2,500 such units per ml.

Chemical reactivity of FA. With the development of a standardized assay it was possible to compare the effects of various treatments on *FA* and bacterial cells. The latter are sterilized by shaking with such agents as chloroform, toluene, alcohol, and formalin. Of these only formalin inactivates *FA*. The bacteria are sterilized by heating at 56°C for 30 minutes. Temperatures of 70°C are necessary for detectable effects on *FA*. It is rapidly inactivated only when 100°C is approached.

FA is quantitatively precipitated from broth by one to two volumes of cold alcohol or half saturated ammonium sulfate. A heavy floc appears in both cases which, for the most part, remains water insoluble; *FA*, however, redisperses.

None of several enzymes tested affected *FA*. They were added directly to the active filtrates and incubated for two hours. The tests included pancreatin (100 mg/ml), trypsin (100 µg/ml), Taka-diastase (100 mg/ml), ribonuclease (10 µg/ml), and desoxyribonuclease (20 µg/ml). The failure of desoxyribonuclease to inac-

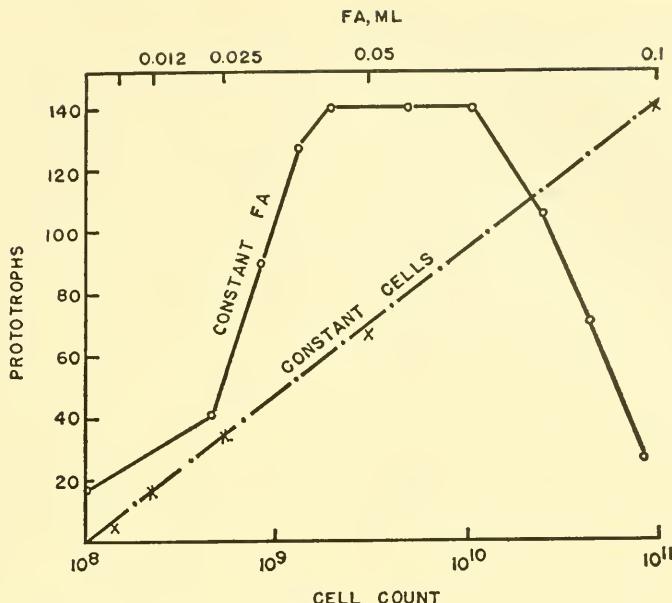


Fig. 1. Assay of *FA*. *FA* (LT-2) and cells (LA-22) were mixed at various dilutions and plated on minimal agar. Prototrophs were counted after 48 hours.

tivate *FA* was of particular interest. Enzymatic activity was verified by testing samples of the reaction mixture for reduction of the viscosity of thymus nucleic acid (kindly supplied by Dr. R. D. Hotchkiss). Similar controls were not done for the other enzymes.

Evocation of FA. The properties of phages latent in *Salmonella* have been summarized by Burnet and McKie (1929) and Boyd (1951). Lysogenic phages, i.e., those obtained from lysogenic bacteria, poorly lyse sensitive cultures and readily provoke secondary resistant lysogenic forms. Visible lysis of sensitive cells is observed only at low multiplicities of infection. With higher multiplicities there is little evidence of lysis. When phage is added to broth cultures, the tube does not clear, and the bacteria grow at a reduced but significant rate. PLT-22 is typical of these phages.

To determine whether PLT-22 was unique in its *FA* evoking activity, a

variety of treatments was applied to resting and growing cells of LT-2 strains. *FA* was not detected in the filtrates of young cultures or after autolysis with benzene, extraction of dried cells, treatment with high concentrations of antibiotics (penicillin, bacitracin, and aureomycin), or complete phage lysis. Dilute antibiotics, lithium chloride, and crystal violet yielded variable *FA*. High activity is most readily detected in the filtrates of cultures treated with lysogenic phages. These results indicate that *FA* is not released by mechanical, chemical, or biological disruption of cells. However, various deleterious agents elicit its appearance in a way that may parallel the action of latent phage. The most effective concentrations of these reagents were those which only slightly inhibited the cells. *FA* has also been detected in aged cultures when autolysis sets in. This may be due to the action of mutant lysogenic phage.

The production of *FA* in response to chemical stimuli has not yet been sufficiently controlled to give consistent yields needed for experimental use. However, when a filtrate containing little or no *FA* is prepared after treatment of LA-2 with such agents as crystal violet or penicillin and reinoculated into broth with LA-2, *FA* is released in large amounts. This procedure has been carried through for five cycles. The apparent regeneration of *FA* was probably due to a lysogenic phage which had been released in the first treatment. The lack of a reliable indicator for this phage has hindered the analysis of this reaction. However, it has been a useful tool for the evocation of *FA* from a single strain without the introduction of extraneous bacteria or viruses.

Morphological and physical studies. *FA* has been quantitatively sedimented and recovered in the Spinco ultracentrifuge at 100,000 G for 30 min. Partial sedimentation occurred in the International centrifuge with multispeed head at 20,000 G. In these prepara-

tions *FA* is, therefore, of more than macromolecular dimensions. Filtration through a series of gradocol membranes (obtained through the courtesy of Dr. S. E. Luria) was also used to estimate the particle size. Ten to twenty per cent of *FA* were retained by a membrane of A.P.D. 420 m μ , seventy per cent at 230 and 170 m μ , and ninety-nine per cent at 120 m μ . These results indicate a particle size slightly less than 0.1 μ (Bawden, 1950).

FA preparations exhibit numbers of small, barely resolvable, granules under the phase contrast microscope. Electron micrographs show granules whose size is in rough agreement with the estimates of *FA* from filtration experiments (figure 2). Some of the granules agglutinated with anti-O serum. Visible floccules which can be removed by centrifugation appeared in the reaction tube. However, the activity remained intact in the supernatant. Upon incubation with anti-serum, some of the granules enlarge and by four hours have attained sizes of 5 to 8 μ (see figure 11, Lederberg

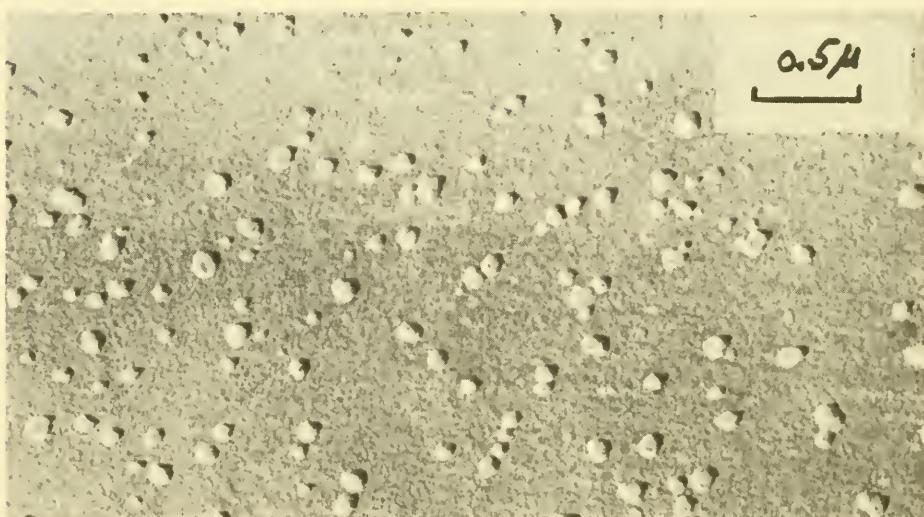


Fig. 2. A partially purified active filtrate, 40,000 X. (Electron micrograph by Dr. Paul Kaesberg.)

et al., 1951). These "large bodies" formed mixed floccules with added bacterial cells.

Treatment of *Salmonella* with *FA*-eliciting phage or penicillin results in the formation of chains after one and one-half hours of incubation, and by three hours only "snakes" with swollen bulbular central portions are present (Fleming et al., 1950). Debris and small granules are also seen. *FA* has also been produced by this time. The supernatants of these cultures were difficult to sterilize by conventional means. Filtration through eight or fourteen pound test Mandler candles resulted in filtrates with a viable count of about 100 per ml. Comparable filtrates of untreated cultures have regularly proven to be sterile. Sintered pyrex "UF" filters were found to be suitable for sterile filtration of active filtrates.

These observations are reminiscent of the L-forms of bacteria particularly as interpreted by Klieneberger-Nobel (1951), Dienes and Weinberger (1951), and Tulasne (1951). There is, however, no evidence of a functional relationship between L-forms and transduction. We have not yet succeeded in obtaining L-colony growth from our cultures that would permit more direct tests, nor have other workers made genetic analyses of L-type growth to fortify speculations on their role in a life cycle.

Sources and range of activity of FA. *FA* has been defined thus far as a specific product of strain LA-2 with the single capacity of transducing a particular mutant of LT-22. However, other direct crosses involving LA-22 had given prototrophs. To determine if *FA* could be obtained from other strains, a simplified test was applied, involving the selection of streptomycin resistant prototrophs, "SRP" (Lederberg, 1951a). SW-435 (LA-22 S^r) was

grown in mixed culture with each of fifty different wild type (streptomycin sensitive prototrophs) *S. typhimurium* strains, and the mixture plated on minimal agar containing 500 mg per L of streptomycin. Twenty-eight of the crosses yielded evident recombinants, showing that *FA* could probably be produced by many strains.

FA has been isolated from each of twenty-five tested strains of *S. typhimurium* when the proper stimulus was found. PLT-22 served for the many strains susceptible to it, which probably explains the success of the SRP crosses, while other lysogenic phages (from the Lilleengen series) stimulated other strains resistant to PLT-22. In general, inoculation of 10^9 cells of *S. typhimurium* and 10^8 to 10^9 particles of a lysogenic phage to which it is susceptible into 10 ml of fresh broth will yield *FA* after four hours of incubation. Penicillin in low concentrations (one to five units per ml) was successful for some cultures.

A demonstration of recombination in *Salmonella* was initially sought and found in terms of the recovery of prototrophs from mixed platings of auxotrophs. A more complete proof of typical sexuality would depend upon the occurrence of new combinations of "unselected markers" (Lederberg, 1947). SW-478 (LA-22 Gal-, Xyl-, Mtl-, S^r) was crossed with SW-414 (LA-2 Gal+, Xyl+, Mtl+, S^s) on EML agar containing one of the various sugars so that one unselected fermentative character could be scored directly on the cross plate. Of some 20,000 prototrophs screened, none differed from SW-478 except in their nutrition. In addition to mutational differences, LA-22 and LA-2 differed intrinsically in ability to utilize malate, alanine, or succinate as the sole carbon source required for growth. All of the prototrophs resembled LA-22. With a

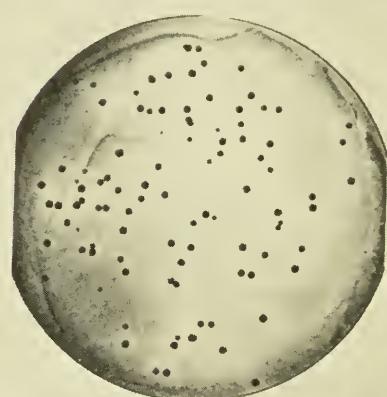
total of eight unselected markers there was no evidence of co-segregation. These experiments were repeated with active filtrates from LT-2 and gave the same result.

Genetic transfers for each of three markers (one nutritional and two fermentative) were observed when experiments were set up in such a way as to select for them. SW-435 (Aux , Gal^- , Xyl^- , S^r) was plated with FA (from LT-2 Prot, Gal^+ , Xyl^+ , S^s) on minimal, EMB galactose and EMB xylose agar. Upon the EMB media

there first appeared a thin film of growth (pink and hence nonfermenting) and then small outcroppings or papillae which fermented the galactose or xylose. These papillae grow quite large (figures 3 and 4) because of their utilization of the sugar when other nutrients are depleted. The xylose-negative mutant gave some papillae due to spontaneous reversion but not enough to interfere with the scoring of the test. The galactose negative mutant is more stable and has only rarely reverted. The number of papillae on



3



4

Figs. 3 and 4. SW-435 plated on EMB galactose agar with heat inactivated (3) and active (4) FA .

EMB or prototrophs on minimal agar (table 2) was approximately the same so that the efficiency of transduction

for different selected characters may be uniform. However, the unselected markers remained unaltered; that is, all

TABLE 2
SW-435 and FA upon different selective media

Medium	<i>FA</i>	Boiled <i>FA</i> (control)
Minimal	120 prototrophs	0 colonies
EMB galactose	114 papillae	0 papillae; film of bacteria
EMB xylose	138 papillae	15 papillae; film of bacteria

Figures are colonies or papillae per plate.

prototroph selections were nonfermenters and the papillae selections acted only upon the one sugar and were auxotrophic. All of the transduced cells were still streptomycin resistant.

The foregoing experiment was repeated on double sugar agar. Individual papillae fermented either galactose or xylose and were all auxotrophic. Because of a slight difference in texture it was possible to differentiate the two kinds of papillae directly on the indicator plate. Entire papillae were picked and transferred to the alternative sugar and to minimal agar. Among the many tested, no mixed papillae were found. Any such could be detected by this rigid selection.

From these experiments, we conclude that an *FA* filtrate has many activities, producing many different transductions (but no more than one per cell) that result in singly transduced clones.

We have observed no linked segregations such as had been found in *E. coli* recombination. The singular activity of *FA* might still be reconcilable with a gametic interpretation if the failure to show linkages were due to

structural differences in the chromosomes of the parents. Alternatively, *FA* might have been considered in terms of a nonspecific mutagen with independent action on different factors. Further experiments have discredited both of these views beyond reasonable doubt.

LT-7 served as an efficient donor and receptor of *FA* and was chosen for the study of the intrastrain transfers and to test these considerations (see table 1 for its markers). To be certain of the source of the *FA* employed, it was prepared (as described previously) without external bacterial or viral influences. *FA* was prepared from SW-184 (prolineless), SW-188 (methionineless), and SW-191 (leucineless). Each preparation was assayed for transduction from auxotrophy to prototrophy of each of the three *LT-7* auxotrophs and *LA-22* (control for the presence of any activity). The preparations had fairly uniform activity on *LA-22*. However, *FA* from each of the three *LT-7* auxotrophs could transduce the other two but not its source culture (table 3). *FA* thus conforms to the genotype of the cells from which it comes. Several galac-

TABLE 3

The effect of FA from LT-7 and its derivatives upon LT-7 derivatives

Cells/FA	LT-7	SW-184	SW-188	SW-191	Boiled FA
SW-184	203	26*	247	253	31*
SW-188	62	76	0	68	0
SW-191	198	210	236	18*	10*
LA-22 (control)	230	242	202	275	0

* Presumably spontaneous reverse mutations.

Figures are transductions from auxotrophy to prototrophy per plate.

tose-negative mutants were obtained in each of the three auxotrophs. None of several thousand transduced prototrophs was galactose positive. *FA*, from SW-184 (prolineless), when

plated with SW-188 (methionineless) on minimal agar supplemented with proline, resulted only in proline independent colonies (prototrophs). Comparable results have been obtained

with each of the three auxotrophs in similar experiments. In the course of transduction, there was no linked segregation or association of these three nutritional markers with each other or with fermentative markers. Streptomycin resistance provided still another marker that remained unaltered in cells transduced for other characters.

Several galactose-negative mutants were transducible to galactose-positive

by *FA* from their parental wild type. *FA* from these mutants gave diverse results. The mutants were never transduced by their own *FA*, but they could be transduced by *FA* from some of the other mutants. These interactions provided a basis for grouping the mutants with respect to allelism or genic identity (table 4).

All of the transductions discussed thus far have been in the direction of mutant to wild type. It is difficult, as

TABLE 4

The effect of FA from several galactose-negative mutants upon these same mutants

Cells/FA	LT-7	Gal-1	Gal-2	Gal-3	Gal-4
Gal-1	+	-†	-	+	+
Gal-2	+	-	-	+	+
Gal-3	+	+	+	-	-
Gal-4	+	+	+	-	-

* Galactose positive papillae produced.

† No more papillae than on control.

a rule, to screen for changes in the other direction owing to the lack of adequate selective methods. This can be done with streptomycin resistance since the wild type condition is sensitive (*S^s*) and the mutant is resistant (*S^r*). Freshly harvested cells were exposed to *FA* from streptomycin resistant and sensitive "parents" and then plated upon EMB galactose. After two hours of incubation (to allow for phenomic lag, Davis 1950a) the plates

were sprayed with a concentrated solution of streptomycin (0.1 g per ml). Table 5 shows that the transduction did occur but only when a streptomycin resistant mutant was the source of the *FA* employed. No associated changes were found. The stability of the transduced cells was verified by tests of many daughter colonies by replica plating (Lederberg and Lederberg, 1952) to normal and streptomycin containing media.

TABLE 5

Comparison of the effect of FA from streptomycin resistant and sensitive cells on sensitive cells

Cells/FA	LT-7 (Gal+, S ^s)	SW-514 (Gal+, S ^r)	SW-191 (Gal-, S ^s)	SW-515 (Gal-, S ^r)
LT-7 (Gal+, S ^s)	0	203 Gal+	0	174 Gal+
SW-191 (Gal-, S ^s)	0	228 Gal-	0	158 Gal-

Figures are the number of streptomycin-resistant colonies per plate.

It is now evident that the particular *FA* for which an assay has been defined is just one of several coexisting functions of a given filtrate. We are entitled to refer to *FA* for any of the genetic factors so far studied, and the range of action of a given filtrate can be designated in the same way as the genotype of the culture from which it is obtained: e.g., *Prot*, *Gal+*, *Xyl+*, *S^r* for SW-514 (figure 5), as well as for the *FA* derived from it. Unless otherwise qualified, however, *FA* will continue to refer to the transduction assayed on LA-22.

Adsorption of FA. The first step in transduction must be the adsorption of *FA* on competent cells. LA-22 was harvested from nutrient agar plates. Aliquots were suspended in one ml of an active filtrate for various intervals. The cells were sedimented and plated on minimal agar to determine the num-

ber of exchanges. After a heat shock at 56°C to destroy any unsedimented cells, the supernatants were assayed with LA-22 for unadsorbed *FA*. Moderate amounts of *FA* were completely adsorbed within the time necessary for centrifugation (15 minutes) and were recovered quantitatively in the precipitated cells.

All tested smooth strains of *S. typhimurium* adsorbed *FA*. Cells of the donor strain adsorbed as efficiently as the others, consistently with the success of intrastrain transfers. Disinfection by boiling or ultraviolet irradiation (to leave an extremely small viable fraction) did not affect adsorption. Rough cultures, selected by aging in broth (Page *et al.*, 1951) did not adsorb. These results indicated that the site of adsorption is heat stable, is not affected by the death of the cell, and may be related to the somatic antigen.

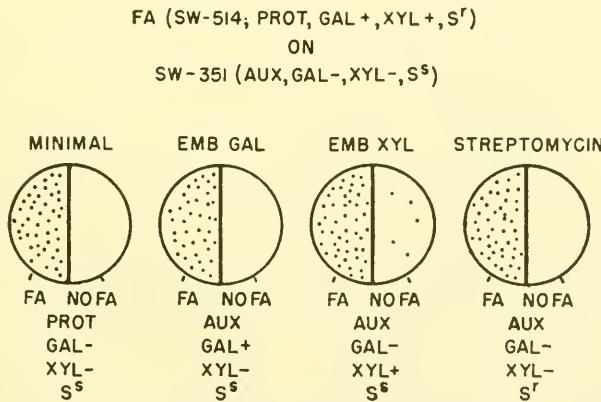


Fig. 5. Multiple potentialities of an active filtrate.

With the amounts previously used, *FA* assays were directly proportional to *FA* concentration. Cells of LA-22 were harvested from nutrient agar. Aliquots containing 10^{10} cells were sedimented in each of ten centrifuge tubes and the supernates discarded. Multiple aliquots of *FA* (one to ten ml) were added and 15 minutes at 37

C allowed for adsorption. Supernates and cells were collected and assayed on EML galactose. No concordant changes (i.e., galactose positive) were observed among the prototrophs. Figure 6 shows that a maximum number of transductions occurred with about eight ml of *FA*. The saturated sediments adsorb no more *FA* from larger

aliquots. Except for a small systematic loss, probably mechanical, all units of *FA* are accounted for either in the supernatant or the sediment.

The interference in adsorption implied by saturation was demonstrated more explicitly in a blocking experiment. SW-188 (methionineless, M-)

was exposed to an excess of *FA* from M- cells for fifteen minutes. *FA* from LT-7 (M+) was then added to the sedimented cells for an additional fifteen minutes before the cells were again sedimented. The M+ *FA* was not bound, nor was the SW-188 transduced. This verifies the blocking con-

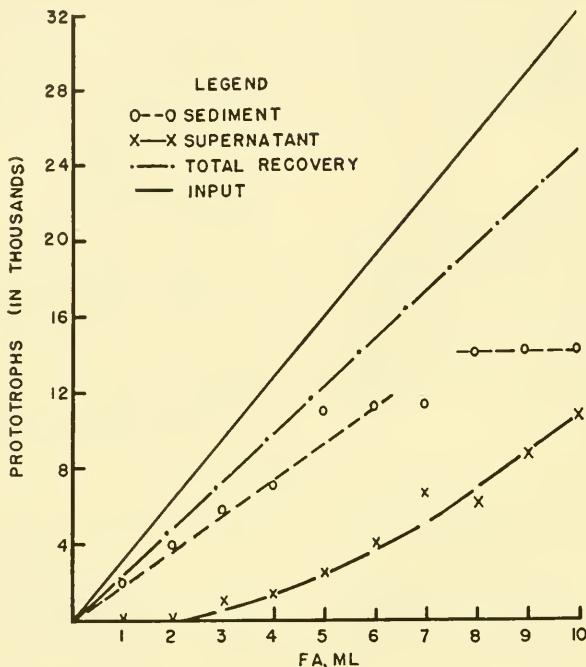


Fig. 6. Adsorption of *FA*. 10^{10} cells of LA-22 were exposed to *FA* (LT-2) for 15 to 30 minutes. Supernatants and sediments were collected after centrifugation and assayed, respectively, for residual *FA* and for transductions already initiated.

cept and indicates that the adsorption is irreversible after the fifteen minutes allowed for saturation.

Since adsorption of *FA* is so rapid it appears safe to assume that the large proportion of the individual bacteria are capable of adsorbing it. We can make an approximate minimum estimate of the number of adsorbable particles per ml of this filtrate by dividing the total number of bacteria by the number of ml required to saturate them (one particle per bacterium);

$10^{10}/8$ or 1.3×10^9 . A maximum number of particles per ml is set by the fact that the active filtrate showed no turbidity as might be expected with more than 10^{11} particles per ml.

So many unverified postulates are required that a detailed discussion of possible models for the kinetics of adsorption would be unprofitable here. It may be pointed out, however, that the low or zero frequency of double exchanges does not imply that one species of *FA* particle excludes an-

other. If most of the bacteria are competent to be transduced, the frequency of a particular transduction will be the probability that any of the particles adsorbed will have a particular effect. Double transductions will occur in the same ratio to single exchanges as the absolute frequency of the latter, and this is too low (ca 10^{-5}) for double exchanges to be detected in our experiments. However, if transduction is limited to a small proportion of competent cells, dual transductions would not have independent probabilities, and further assumptions such as mutual exclusion would be required to account for the low frequency of observed dual events.

The following picture appears to be most consistent with the observations to date. An active filtrate contains a population of numerous species of granules, each corresponding to a genetic effect although some may be intrinsically inert. Each bacterium may absorb a limited number of particles, in the possible range from one to perhaps one hundred. Each adsorbed particle has a fixed, independent probability of exerting its particular transductive effect. The low frequency of single, and particularly of double transductions, is limited by the total number of particles that may be adsorbed as well, perhaps, as by the low probability that an adsorbed particle will complete its effect.

Serial transduction. Dual transduction has never been observed in a single experiment. That this is due to the considerations described previously rather than some intrinsic limitation is shown by serial transfers. Once a cell has been transduced it can be grown out, reexposed to FA, and selected for other changes. SW-351 (Aux, Gal-, Xyl-) has been serially transduced from auxotrophy to prototrophy, from galactose negative to positive, and

from xylose negative to positive. The order in which these transfers were accomplished made no difference. There was no loss of efficiency with the iterated transductions as compared to the single transduction of SW-351 for any of the characters.

Specificity of adsorption of FA. The adsorption experiments had indicated a correlation of adsorptive ability and immunological specificity. Preliminary experiments with some dozen *Salmonella* serotypes confirmed and narrowed this correlation to the presence of somatic antigen XII. Broth cultures of the serotypes to be tested were sedimented and one ml of FA was added. Adsorption proceeded for fifteen minutes, and then the reaction tubes were heat shocked at 56°C for one hour to sterilize the cells. Preliminary experiments with known adsorbing cells had shown that FA once adsorbed was not eluted by this procedure. The mixtures were assayed on LA-22 for free FA. Some fifty different serotypes have been tested in this manner. Although some types with XII are inert, none of the types without XII adsorbed. This correlation is maintained with the "*Salmonella coli*" types. The XII carrying strains that adsorbed were: *S. paratyphi* B, *S. typhimurium* (25 strains), *S. stanley*, *S. heidelberg*, *S. chester*, *S. san-diego*, *S. abortus-ovis*, *S. typhi* W, *S. typhi* V, *S. enteritidis*, *S. moscow*, *S. blegdam*, *S. eastbourne*, *S. sendai*, *S. abony*, *E. coli* 3, *E. coli* 4, *S. kaapstad*, *S. salinatis*, *S. pullorum*, and *S. gallinarum*. The following XII types did not adsorb: *S. paratyphi* A and *S. abortus-bovis*, presumably owing to the absence of the XII₂ component. The nonadsorbing, non-XII types tested were: *S. typhimurium* (rough variant), *S. cholerae-suis*, *S. newport*, *S. london*, *S. senftenberg*, *S. aberdeen*, *S. poona*, *S. worthington*, *S. hvidtingfoss*, *S. ken-*

tucky, *S. wichita*, *S. urbana*, *S. habana*, *S. altendorf*, *S. vejle*, *S. montevideo*, *E. coli* 1, *E. coli* 2, *E. coli* 5, *E. coli* K-12, *S. bonariensis*, *S. florida*, and *S. medelia*.

Inter-type transductions. It is not known whether the adsorption of *FA* is sufficient to indicate susceptibility to genetic transfer, but preliminary data identify a possible receptor group, among which inter-type transductions may be possible.

S. typhi and *S. typhimurium* differ in a number of cultural and serological characters. The latter ferments both arabinose and rhamnose while the former does not ferment and is inhibited by either of these sugars. *S. typhi* Watson V was exposed to *FA* from *S. typhimurium* and inoculated into Durham fermentation tubes containing one per cent of either sugar in nutrient broth. After 24 hours a more luxuriant growth appeared in the *FA* treated cultures, and acid was produced by 48 hours. From these tubes cultures were isolated that differ from *S. typhi* only in their ability to ferment these sugars. The control cultures, without *FA*, show little evidence of growth and no evidence of fermentation. Although *S. typhimurium* produces gas from rhamnose and arabinose, these new forms remain typically anaerogenic. The experiment has also been conducted on agar. Treated cells were plated on EMB arabinose and EMB rhamnose. *S. typhi* occasionally mutates to a noninhibited form (Kristensen, 1948) which was represented by white papillae which were observed on both the experimental and control plates. However, the purple (fermenting) papillae were observed only on the experimental plates. Culturally they resembled the fermenting strains isolated after transduction in broth. These results have been repeated with two other strains of *S. typhi*.

Using a streptomycin resistant mutant of *S. typhimurium* as the source of *FA*, it has been possible to transfer this character to *S. typhi*. Attempts to produce aerogenic fermentation of glucose by *S. typhi* by treatment with *FA* have all met with failure, possibly owing to insufficiently selective conditions to detect cells transduced for this character.

S. typhi is antigenically characterized IX, XII: *d*, — (monophasic) while *S. typhimurium* is I, IV, V, XII: *i* — 1, 2, 3. *S. typhi* was exposed to *FA* from *S. typhimurium*, and transduction of the flagellar antigen was selected for. A tube based upon the mycological growth tube (Ryan *et al.*, 1943) was half filled with soft agar containing diluted anti-*d* serum (1/200 of serum titrating to 1/5,000). The cells were heavily inoculated at one side of the tube and watched for migration. In one experiment, two out of four experimental tubes showed migration while the three control tubes showed complete fixation of the inoculum. There was a sharp delineation between the migrating cells and the fixed inoculum. The former were fished from the uninoculated end of the tube and tested culturally and serologically. Both of the isolates culturally resembled *S. typhi*. One of them reacted with anti-*i* serum while the other did not react with either *S. typhi* or *S. typhimurium* flagellar antiserum and was diagnosed as a *j* phase (Kauffmann, 1936). The analysis of these two strains was confirmed by Dr. P. R. Edwards. Transduction of the *i* antigen was obtained from twelve of thirty-one tested inocula of 10^8 *FA* saturated *S. typhi* cells. "*j*" phases have appeared occasionally in both experimental and control tubes. No *i* phases were detected in 50 control tests without *FA*. The complete antigenic analysis of the "hybrids" is IX, XII: *i*, —.

Unlike *S. typhimurium*, from which the *i* flagellar phase was derived, phasic variation has not been found in these "hybrids". Experiments are now in progress seeking transduction of other flagellar and somatic antigens.

The transduced cell. Prototrophs produced by transduction [*FA* (LA-2) on LA-22] have been tested for their stability both in vegetative reproduction and further transduction. After isolation from the experimental plate they were purified by streaking. Five single colonies were grown in complete broth and plated. Two hundred colonies from each were picked and retested on minimal agar; all were prototrophic. The transduced culture was reexposed to *FA*, and another change was selected (galactose negative to positive). Of some 1,500 colonies tested by replica plating, all retained the initial transduction to prototrophy.

The transduced culture does not release *FA* during its growth nor is *FA* obtainable from it by any other means than those employed for the parent culture. Some difficulty has been encountered in this respect with the products of intrastrain transduction. They were all resistant carriers of the phage associated with active filtrates and some new phage was needed to evoke *FA* from them. Phage resistance also reduces the efficiency of iterated transduction, presumably because of impaired adsorption of *FA*.

Spontaneous reverse-mutations regain the ability to transduce their mutant parents as do transduced revertions. That is, when a cell goes from A- to A+ by either means, it can again produce A+ agent. Mutation in free *FA* has not yet been studied.

The relationship between bacteriophage and FA. Several recent convergent lines of evidence point to the identity of *FA* particles and bacteriophage.

FA and phage have a common filtration end point; ninety-nine per cent of both are retained by a membrane of A.P.D. 120 m μ . They have a common specificity of adsorption on *Salmonella* serotypes, correlated with somatic antigen XII. In adsorption on *S. typhimurium* both reach saturation at the same point, and the phage to *FA* ratio remains constant. During the course of purification, *FA* and phage remain together. In short term experiments, *FA* and phage are released simultaneously from phage infected bacteria. Electron micrographs show a morphological similarity of particles of proper size.

That the phage particle can be only a passive carrier of the transductive genetic material is shown by the following experiments. From single phage particles grown on bacterial cells there are obtained high titered phage and a population of *FA* encompassing the entire genotype of the parental cells but capable of only one transduction per bacterial cell. Single phage particles, from this filtrate, can be grown on bacterial cells from the same original parent but of different genotype. The *FA* produced is comparable to the genotype of the secondary donor.

In the section on the evocation of *FA*, mention was made of the apparent regeneration of *FA* by transfer. This was explained as being due to the association of *FA* with phage which served to continuously stimulate its production. To test this, A-, B+, C+ cells were treated with penicillin. The filtrate was transferred with the same cells to yield *FA* (A-, B+, C+) and a phage which could be assayed on these same cells. When added to A+, B-, C+ cells (from the same original parent), the *FA* obtained was A+, B-, C+. All of the B+ agent was adsorbed and lost, and agents paralleling the genotype of the B- cells obtained.

FA had not propagated as such but rather was associated with the necessary stimulus for further production, the phage.

DISCUSSION

Genetic exchange in *S. typhimurium* is mediated by a bacterial product which we have called *FA* (filtrable agent). An individual active filtrate can transfer (transduce) many hereditary traits from one strain to another. Although the total activity of this filtrate encompasses the genotype of its parental culture, each transduction transmits only a single trait per bacterium. This contrasts with genetic exchange in *E. coli*, strain K-12, where there is unrestricted recombination of the several markers that differentiate two parental lines.

FA may be considered as genetic material which enters the fixed heredity of the transduced cell. We may ask whether this transfer is a simple super-addition or a substitutive exchange and replacement of the resident genetic factors. If streptomycin resistance is a recessive mutation, as inferred from studies of heterozygous diploids in *E. coli* (Lederberg, 1951b), the transduction of resistance disqualifies the simple addition mechanism.

Two aspects of *FA* must be carefully distinguished: the biological nature of the particles themselves and their genetic function. There is good reason to identify the particle with bacteriophage. Nevertheless, the phage particle would function as a passive carrier of the genetic material transduced from one bacterium to another. This material corresponds only to a fragment of the bacterial genotype. For example, when *FA* from a marked prototroph is plated with an auxotroph on minimal agar, the genotype of the presumed "donor nucleus" is not observed among the transduced proto-

trophs. The hypothesis of *FA* as a genetic complex rather than a unit might be maintained if the singular effects produced depended on a small chance of release of any particular activity from a complex particle or on some localized nonheritable happenstance in the cell that ordinarily left only one function sensitive to transduction. Still the originally singly transduced cell develops as an isolated clone. Since the clone is composed of some 10^7 bacteria, one might expect that a complex residuum of an *FA* particle, if viable, would transduce some one of the daughter cells for another character during the growth of the clone. However, each *FA* particle produces only a single transduced clone. This speaks for the simplicity of its constitution as well as of its genetic effect.

When LA-22 is transduced from auxotrophy (phenylalanineless and tyrosineless; tryptophanless) to prototrophy, we have an apparent dual change. If this mutant is plated on minimal agar supplemented with phenylalanine and tyrosine, it occasionally reverts to the first step auxotrophic condition. However, when LA-22 is transduced on this medium, no more first step auxotrophs are found than can be explained by spontaneous reversion. The majority of the selected colonies are prototrophs. We have not been able to affect more than one trait in any other inter- or intra-strain transductions. It seems likely that the nutrition of LA-22 was determined by two successive mutations at the same genetic site. Davis' (1951) scheme for aromatic biosynthesis corroborates this notion. Although the mutant LA-22 can revert spontaneously to an intermediate allele, transduction brings about a substitution of the wild type gene for full synthesis.

The most plausible hypothesis for

the *FA* granules is that they are a heterogeneous population of species each with its own competence—in other words, each carries a "single gene" or small chromosome fragment.

Regardless of the nature of the *FA* particles, some mechanism must be postulated for the introduction of the transduced genetic material to the fixed heredity of the recipient cell. Muller's (1947) analysis of type transformation in the pneumococcus is apropos here: ". . . there were, in effect, still viable bacterial chromosomes, or parts of chromosomes, floating free in the medium used. These might, in my opinion, have penetrated the capsuleless bacteria and in part taken root there, perhaps, after having undergone a kind of crossing-over with the chromosomes of the host."

In a preliminary report on the *Salmonella* recombination system (Lederberg *et al.*, 1951) it was suggested that *FA* might be related to bacterial L-forms (Klieneberger-Nobel, 1951). The occurrence of swollen "snakes", filtrable granules, and large bodies in response to certain agents is characteristic both of *FA* and L-forms. Except for the suggestion of viable filter passing granules we have not repeated the reported cycles. The visible agglutinable granules and the antiserum-induced swollen form are not necessary for *FA* activity. However, this failure to fit all of the elements to a simple scheme may be due to a system more complex than we are now aware.

The bacteriological literature has numerous reports of results which might be interpreted as transduction (see reviews by Luria, 1947, and Lederberg, 1948). These experiments have been criticized or neglected because of difficulties in their reproduction and quantitization but might now be reinvestigated in light of the findings presented. A citation of some of

the more pertinent ones should suffice at this time. Wollman and Wollman (1925) reported the acquisition of *Salmonella* immunological specificity by *E. coli* via filter passing material. Similar material (which can be obtained by phage lysis) has been implicated in the change of penicillin resistant staphylococci and streptococci to relative penicillin sensitivity (Vourekas, 1948; George and Pandalai, 1949). *Shigella paradyssenteriae* (Weil and Binder, 1947) acquired new immunological specificity when treated with extracts of heterologous types. Boivin (1947) reported a similar change in *E. coli*. Unfortunately his strains have been lost and confirmation is impossible. Bruner and Edwards (1948) in a report of variation of somatic antigens of *Salmonella* grown in the presence of specific serum commented on the possibility that bacterial products dissolved in the serum were responsible for the changes.

These systems, provocative as they are, are insufficiently documented for detailed comparison with *Salmonella* transduction. The transformations in the pneumococcus (Avery *et al.*, 1944; McCarty, 1946) and *Hemophilus influenzae* (Alexander and Leidy, 1951) have been studied more completely.

The genetic "transformation" of the capsular character of the pneumococcus depends on a specific bacterial product (pneumococcus transforming principle, *PTP*). Originally interpreted as a directed mutation, it is now regarded as a variety of genetic exchange (Ephrussi-Taylor, 1950). Thus far transformations have been achieved for the full capsular character (Griffith, 1928), a series of intermediate capsular characters (Ephrussi-Taylor, 1951), M protein character (Austrian and MacLeod, 1949), and penicillin resistance (Hotchkiss, 1951). As in *Salmonella* each character is trans-

formed independently. However, there are several differences between the two systems. *FA* must be evoked while the *PTP* is extractible from healthy cells. The resistance of *FA* to various chemical treatments has given only negative evidence of its chemical nature. The role of deoxyribonucleic acid in the *PTP* was verified by its inactivation by deoxyribonucleic acidase. Retention of activity by gradocol membranes has given comparable estimates for the size (about 0.1 μ) of the *FA* particles affecting two different characters. On the other hand, while the particle size of the *PTP* has been variously estimated from an average centrifugal mass of 500,000 (Avery *et al.*, 1944) to an ionizing irradiation sensitive volume equivalent to a molecular weight of 18,000,000 with high asymmetry (Fluke *et al.*, 1951), it is considerably smaller than the *FA* particle. Pneumococci must be sensitized by a complex serum system for adsorption of *PTP*. The low but poorly determined frequency of transformations has been thought to be due to the low competence of the bacteria. In the absence of adsorption experiments a system similar to *Salmonella* has not been ruled out. Important information is still lacking in both systems and time may resolve these apparent differences.

The relationship of transduction in *Salmonella* to sexual recombination in *E. coli* is obscure. Transduction has not been found in crossable *E. coli* nor sexual recombination in *Salmonella*. These genera are extremely closely related taxonomically but seem to have entirely different modes of genetic exchange.

Sexual recombination was first demonstrated in *E. coli*, strain K-12. With the development of an efficient screening procedure, two to three per cent of *E. coli* isolates were proved to cross with strain K-12 (Lederberg, 1951a).

The agent of recombination in *E. coli* is almost certainly the bacterial cell. The cells apparently mate, forming zygotes from which parental and recombinant cells may emerge following meiosis, in which linkage is a prominent feature (Lederberg, 1947). The combination of genomes within a single cell has been confirmed by the exceptional occurrence of nondisjunctions which continue to segregate both haploid and diploid complements (Zelle and Lederberg, 1951). Although lysogenicity plays a critical role in transduction in *Salmonella*, all combinations of lysogenic and nonlysogenic cultures of *E. coli* cross with equal facility (Lederberg, E. M., 1951).

Owing to the lack of recombination of unselected markers, transduction is a less useful tool than sexual recombination for certain types of genetic analysis. However, as *FA* may correspond to extracellular genetic material, such problems as gene reproduction, metabolism, and mutation may be more accessible to attack. Sexual systems usually provide for the reassortment of genetic material and give an important source of variation for the operation of natural selection in organic evolution. Both sexual recombination and transduction, because of their low frequency, allow only limited gene interchange in bacteria. Transductive exchange is limited both in frequency and extent.

It is too early to assess the role that transduction may have played in the development of the immunologically complex *Salmonella* species. White (1926) speculated that the many serotypes evolved by loss variation from a single strain possessing all of the many possible antigens. Bruner and Edwards (1948) obtained specific examples of loss variation with contemporary species. Transduction provides a mechanism for transfer of some of the

variation developed spontaneously and independently between the "descending" lines. The genus *Salmonella* includes a group of serotypes which share a receptor for *S. typhimurium FA*. Other receptor groups have yet to be sought. Within such groups it should be possible to evolve in the laboratory other new serotypes comparable to the antigenic hybrid of *S. typhi* and *S. typhimurium*.

Several different bacterial genera have been intensively studied with regard to modes of genetic exchange. Each of the several known systems differs in details that enlarge our notions of bacterial reproduction and heredity.

ACKNOWLEDGMENTS

The authors are indebted to a number of workers cited in the text for providing cultures and other materials. They are especially obligated to Dr. P. R. Edwards, Public Health Service Communicable Diseases Center, Chamblee, Georgia, for patiently providing innumerable cultures, sera, antigenic diagnoses, and counsel.

SUMMARY

When *Salmonella typhimurium* is grown in the presence of a variety of mildly deleterious agents, especially weakly lytic phages, it produces a filtrable agent (*FA*) capable of transferring hereditary traits from one strain to another.

Individual filtrates may transduce many different traits, but no more than one in a single bacterium. The activities of a filtrate parallel the characteristics of the donor cells. Nutritional, fermentative, drug resistance, and antigenic characters have been transduced. The new characters are stable after many generations of subcultures.

FA is resistant to such bacterial disinfectants as chloroform, toluene, and alcohol and to such enzymes as pan-

creatin, trypsin, ribonuclease, and desoxyribonuclease. The size of the *FA* particle, as determined by filtration through gradocol membranes, is about 0.1 micron. Adsorption of *FA* is rapid and, among various serotypes tested, is correlated with the presence of somatic antigen XII.

The maximum frequency of transduction for any one character has been 2×10^{-6} , a limit set by saturation during adsorption. Some inter-type transfers have been observed. For example, the *i* flagellar antigen from *Salmonella typhimurium* has been transduced to *S. typhi* to give a new serotype: IX, XII; *i*, -. Genetic transduction in *Salmonella* is compared and contrasted with "type transformation" in *Hemophilus* and the pneumococcus and with sexual recombination in *Escherichia coli*.

REFERENCES

- Alexander, Hattie E., and Leidy, Grace 1951 Determination of inherited traits of *H. influenzae* by desoxyribonucleic acid fractions isolated from type-specific cells. *J. Exptl. Med.* 93:345-359.
- Austrian, R., and MacLeod, C. M. 1949 Acquisition of M protein through transformation reactions. *J. Exptl. Med.* 89:451-460.
- Avery, O. T., MacLeod, C. M., and McCarty, M. 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types. *J. Exptl. Med.* 79:137-158.
- Bacon, G. A., Burrows, W. W., and Yates, Margaret 1951 The effects of biochemical mutation on the virulence of *Bacterium typhosum*: the loss of virulence of certain mutants. *Brit. J. Exptl. Path.* 32:85-96.
- Bawden, F. C. 1950 Plant viruses and virus diseases. Chronica Botanica Co., Waltham, Mass.
- Boivin, A. 1947 Directed mutation in colon bacilli, by an inducing principle of desoxyribonucleic nature: its meaning for the general biochemistry of heredity. *Cold Spring Harbor Symposia Quant. Biol.* 12: 7-17.
- Boyd, J. S. K. 1950 The symbiotic bacteriophages of *Salmonella typhimurium*. *J. Path. Bact.* 62:501-517.

- 1951 Observations on the relationship of symbiotic and lytic bacteriophage. *J. Path. Bact.* 63:445-457.
- Bruner, D. W., and Edwards, P. R. 1948 Changes induced in the O antigens of *Salmonella*. *J. Bact.* 55:449.
- Burnet, F., and McKie, Margot 1929 Observations on permanently lysogenic strain of *B. enteritidis* Gaertner. *Australian J. Exptl. Biol. Med. Sci.* 6:276-284.
- Davis, B. D. 1950a Studies on nutritionally deficient bacterial mutants isolated by means of penicillin. *Experientia* 6:41-50.
- 1950b Nonfiltrability of the agents of recombination in *Escherichia coli*. *J. Bact.* 60:507-508.
- 1951 Aromatic biosynthesis. III. Role of *p*-aminobenzoic acid in the formation of vitamin B₁₂. *J. Bact.* 62:221-230.
- Dienes, L., and Weinberger, H. J. 1951 The L forms of bacteria. *Bact. Revs.* 15:245-288.
- Ephrussi-Taylor, Harriett 1950 Heredity in pneumococci. *Endeavor* 9:34-40.
- 1951 Genetic aspects of transformations of pneumococci. *Cold Spring Harbor Symposia Quant. Biol.* 16:445-456.
- Fleming, A., Vourekas, Amalia, Kramer, I. R. H., and Hughes, W. H. 1950 The morphology and motility of *Proteus vulgaris* and other organisms cultured in the presence of penicillin. *J. Gen. Microbiol.* 4: 257-269.
- Fluke, D. F., Drew, R. M., and Pollard, C. 1951 The effect of ionizing radiations on the transforming factor of pneumococci. *Science* 114:480.
- George, M., and Pandalai, K. M. 1949 Sensitization of penicillin resistant pathogens. *Lancet* 256:955-957.
- Griffith, F. 1928 The significance of pneumococcal types. *J. Hyg.* 27:113-159.
- Hotchkiss, R. D. 1951 Transfer of penicillin resistance in pneumococci by the desoxyribonucleate derived from resistant cultures. *Cold Spring Harbor Symposia Quant. Biol.* 16:457-462.
- Kauffmann, F. 1936 Ueber die diphasische Natur der Typhusbacillen. *Z. Hyg. Infektionskrankh.* 119:104-118.
- Klieneberger-Nobel, Emma 1951 Filterable forms of bacteria. *Bact. Revs.* 15:77-103.
- Kristensen, M. 1948 Mutative bacterial fermentation. *Acta Path. Microbiol. Scand.* 25:244-248.
- Lederberg, Esther M. 1951 Lysogenicity in *E. coli* K-12. *Genetics* 36:560.
- Lederberg, J. 1947 Gene recombination and linked segregations in *Escherichia coli*. *Genetics* 32:505-525.
- 1948 Problems in microbial genetics. *Heredity* 2:145-198.
- 1950 Isolation and characterization of biochemical mutants of bacteria. *Methods in Medical Research* 3:5-22.
- 1951a Prevalence of *Escherichia coli* strains exhibiting genetic recombination. *Science* 14:68-69.
- 1951b Streptomycin resistance: a genetically recessive mutation. *J. Bact.* 61: 549-550.
- Lederberg, J., and Lederberg, Esther M. 1952 Replica plating and indirect selection of bacterial mutants. *J. Bact.* 63:399-406.
- Lederberg, J., and Zinder, N. 1948 Concentration of biochemical mutants of bacteria with penicillin. *J. Am. Chem. Soc.* 70: 4267.
- Lederberg, J., Lederberg, Esther M., Zinder, N. D., and Lively, Ethelyn R. 1951 Recombination analysis of bacterial heredity. *Cold Spring Harbor Symposia Quant. Biol.* 16: 413-443.
- Lilleengen, K. 1948 Typing *Salmonella typhimurium* by means of bacteriophage. *Acta Path. Microbiol. Scand. Suppl.* 77.
- Luria, S. E. 1947 Recent advances in bacterial genetics. *Bact. Revs.* 11:1-40.
- McCarty, M. 1946 Chemical nature and biological specificity of the substance inducing transformations of pneumococcal types. *Bact. Revs.* 10:63-71.
- Muller, H. J. 1947 The gene. *Proc. Roy. Soc. London, B*, 134:1-37.
- Page, L. A., Goodlow, R. J. and Braun, W. 1951 The effects of threonine on population changes and virulence of *Salmonella typhimurium*. *J. Bact.* 62:639-647.
- Plough, H. H., Miller, Helen Y., and Berry, Marion E. 1951 Alternative amino acid requirements in auxotrophic mutants of *Salmonella typhimurium*. *Proc. Natl. Acad. Sci., U. S.* 37:640-644.
- Ryan, F., Beadle, G., and Tatum, E. 1943 The tube method of measurement of growth rate of *Neurospora*. *Am. J. Botany* 30:784-799.
- Tatum, E. L., and Lederberg, J. 1947 Gene recombination in the bacterium *Escherichia coli*. *J. Bact.* 53:673-684.
- Tulasne, R. 1951 Les formes L des bactéries. *Revue Immunol.* 15:223-251.
- Vourekas, Amalia 1948 Sensitization of penicillin resistant bacteria. *Lancet* 254:62-65.

- Weil, A. J., and Binder, M. 1947 Experimental type transformation of *Shigella paradyenteriae* (Flexner). *Proc. Soc. Exptl. Biol., N. Y.* 66:349-352.
- White, P. B. 1926 Further studies of the *Salmonella* group. *Med. Res. Council (Britain), Spec. Rept. Ser.* no. 103.
- Wollman, E., and Wollman, E. 1925 Sur la transmission parahéreditaire de caractères chez les bactéries. *Compt. rend. soc. biol., Paris* 93:1568-1569.
- Zelle, M. R., and Lederberg, J. 1951 Single cell isolations of diploid heterozygous *Escherichia coli*. *J. Bact.* 61:351-355.



Molecular Structure of Nucleic Acids

A Structure for Deoxyribose Nucleic Acid

J. D. WATSON and F. H. C. CRICK

Reprinted by authors' and publisher's permission from *Nature*, vol. 171, no. 4356, 1953, pp. 737-738.

Although I remarked earlier (page 194) that it is difficult to be sure which of the current and recent papers are likely to become classics, this paper by Watson and Crick is certainly off and running with the best of them. The hypothetical structure of DNA, and from this of course the hypothetical structure of a self-reproducing molecule, has captured the imagination of geneticists as few other ideas ever did. The "Watson-Crick Model" has found its way into popular articles, textbooks, and lectures in genetics courses more rapidly than is usual. Directions for construction of a model for high-school biology courses have appeared in the American Biology Teacher. This paper is the initial report of one of the outstanding achievements of modern genetics.

WE WISH TO SUGGEST A STRUCTURE for the salt of deoxyribose nucleic acid (D. N. A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey (1953). They kindly made their manuscript available to us in ad-

vance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it

is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

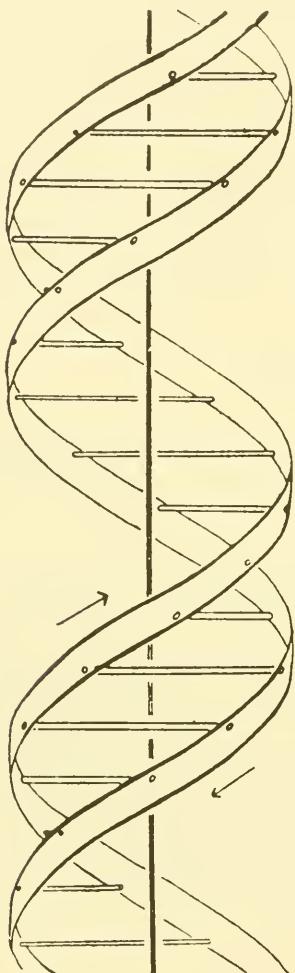


Fig. 1. This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the axis.

Another three-chain structure has also been suggested by Fraser (in the

press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β -D-deoxyribofuranose residues with 3', 5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's (1952) model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration,' the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the

bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-coordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally (Chargaff; Wyatt, 1952) that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data (Astbury, 1947; Wilkins and Randall, 1953) on deoxyribose nucleic

acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

REFERENCES

- Pauling, L. and Corey, R. B. *Nature*, 171: 346, 1953; *Proc. U. S. Nat. Acad. Sci.* 39:84, 1953.
- Furberg, S., *Acta Chem. Scand.* 6:634, 1952.
- Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta* 9:402, 1952.
- Wyatt, G. R., *J. Gen. Physiol.* 36:201, 1952.
- Astbury, W. T., *Symp. Soc. Exp. Biol.* 1, *Nucleic Acid*, 66 (Cambridge Univ. Press, 1947).
- Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta* 10:192, 1953.



The Gene

L. J. STADLER

Reprinted by publisher's permission from *Science*, vol. 120, 1954, pp. 811-819.

This paper by Stadler is interesting in several respects. As the editors of Science commented in a footnote to the original paper, "Dr. Stadler, before his death on 12 May, asked that this paper be sent to Science. It is the valediction, and a remarkable one, of a great geneticist." It can be considered the distillate of Stadler's long and successful years of genetic investigation, and is valuable even if it were no more than that.

But it is considerably more. For Stadler registers here his objections to the kind of research that has been done on the gene and its actions, points out some of the basic fallacies of current concepts in his opinion, and indicates ways and means through which a changed viewpoint might be developed. It is too early to say what impact the paper will have on genetic research. Certainly some of the suggestions made by Stadler cannot be implemented with the research tools currently in use. But his ideas are provocative, and his recommendations deserve to be tested. It would be good for genetics, and equally so for all other fields of biology, if more of those workers with long years of experience would set down their accumulated concepts and research ideas as retirement approaches, as did Stadler. All too often a man's accumulated knowledge dies with him.

THE CENTRAL PROBLEM OF BIOLOGY is the physical nature of living substance. It is this that gives drive and zest to the study of the gene, for the investigation of the behavior of genic substance seems at present our most direct approach to this problem.

Current knowledge of the behavior of living cells presents two striking pictures. The first is the almost incredibly delicate balance of chemical reactions occurring in the living cell, by which energy is made available and

by which the syntheses proceed that provide the materials for growth. The second is the behavior of the genic substance, which apparently guides these reactions. It is carried in the chromosomes in fine strands, which together make up only a minute portion of the substance of the cell. These strands are differentiated along their length into hundreds of segments of distinctive action, and, therefore, presumably of distinctive constitution, which we speak of as the genes. The

genic substance is reduplicated in each cell generation. Its distinctive segments, in many known cases, determine whether or not a specific chemical reaction will occur, presumably, in some cases at least, by determining the production of a specific catalyst.

The great bulk of the substance of the cell apparently consists of materials produced by the aforementioned guided reactions. The nature and behavior of these materials, so far as we know them, do not require the assumption that they have properties essentially different from those of non-living matter.

The genic substance, on the contrary, appears to have properties quite different from those with which we are familiar from our knowledge of the physical science of nonliving matter. Modern physical science gives us no model to explain the reduplication of the gene-string in each cell generation, or to explain the production of effective quantities of specific enzymes or other agents by specific genes. The precise pairing and interchange of segments by homologous gene-strings at meiosis also suggest novel physical properties of this form of matter. These facts indicate that a knowledge of the nature and properties of the genic substance might give clues to the distinctive physical mechanisms of life.

The difficulties in the study of the genic substance are obvious. It cannot be isolated for chemical analysis or pure culture. The possibility of direct analysis of specific segments or individual genes is, of course, even more remote. The properties of the genes may be inferred only from the results of their action.

Furthermore, a critical study of the effects of a single gene may be made only by comparing individuals wholly comparable in genotype except for a difference in the one gene concerned.

This means that gene mutations are essential for such comparisons, since it is only by gene mutation that we can identify individuals differing only by the effects of a single gene. The prospect of determining the properties of the gene is, therefore, dependent upon the development of valid methods for the study of gene mutation.

It is appropriate to cite here the monumental contributions of H. J. Muller to the investigation of this problem. More than 30 years ago he recognized clearly the unique significance of gene mutation in the study of the physical nature of life (1) and boldly attacked the imposing technical problems that blocked its experimental investigation.

The difficulties of analysis that have been mentioned are not different in kind from those involved in other problems in which the properties of hypothetical elements must be inferred from their effects—for example, in the problems of molecular or atomic structure. In such studies, the investigator proceeds by constructing the simplest model that will fit the known facts and then attempting to apply every significant experimental test of the predictions that may be made from the model. By a series of successive approximations, the model finally evolves to a form that seems to provide the most plausible mechanism for the behavior observed. The study of the physical nature of the gene from purely genetic evidence is closely comparable to this.

These difficulties of analysis are mitigated in some degree by the possibility of parallel investigation of certain problems of mutation through direct observation of the chromosomes. Although the gene-string itself is below the limit of microscopic visibility, its behavior is such that it provides a

visible shadow, so to speak, in the chromosome. Some alterations of the gene-strings are readily detectable by visible alteration of the chromosomes. The cytogenetic analysis of individual mutations provides a wholesome check on hypotheses derived from the statistics of mutation frequencies.

An illuminating example of this is afforded by certain interpretations of the evidence on mutation rate as affected by x-ray treatment and by temperature. At an early stage in the study of x-ray-induced mutations, Delbrueck (2) constructed a tentative "atomic physics model" of the gene, as inferred from the frequency of point mutations observed under varying physical conditions. This has become widely known through its application and discussion in the engaging little book *What Is Life?* (3), published several years later by the eminent theoretical physicist, Erwin Schrödinger.

In this view, the gene is considered a molecule, and the observed mutations are considered to represent its transitions from one stable state to another, as a result of thermal agitation or the absorption of radiant energy. The linear-dosage curve and the constancy of mutation yield, regardless of variation in the time factor, show that the x-ray-induced mutations result from single "hits"; the constant proportionality of mutation yields to ionization, regardless of variation in wavelength, shows that the unit "hit" is an ionization. Calculation of the volume within which these hits must occur to account for the mutations observed provides a basis for estimating the average size of the gene-molecules postulated. This turns out to be of the order of 1000 atoms. The relative frequency of spontaneous mutations at different temperatures permits the calculation of the activation energy required for the occurrence of a muta-

tion, which turns out to be about 1.5 ev. Unstable genes are assumed to have correspondingly lower activation energies, and the fact that temperature affects their mutation rate less than that of normally stable genes is in agreement with expectation on this basis. The energy spent in one ionization is about 30 ev, and it is therefore to be expected that irradiation will cause the mutation of any of the genes, regardless of their relative stability under normal conditions. The proportional increase in mutation rate will, therefore, be much less for genes distinctly unstable at ordinary temperatures than for genes of normal stability. These expectations also are realized.

This is an impressive picture, but it has been evident for many years that it has no valid relationship to the experimental data from which it was derived. The detailed analysis of individual cases among the x-ray-induced mutations has shown clearly that many of these result not from a structural change in a gene but from some alteration external to the gene, such as physical loss or rearrangement of a segment of the gene-string. We have no basis for estimating the proportion of such extragenic mutations among the total of mutations observed and no ground for assuming that this proportion is the same among the mutations observed under the various experimental treatments.

The basis of the model is the assumption that the statistics of observed mutation are in fact the statistics of structural alteration of the molecules that constitute the gene-string. The investigations of specific mutations contradict this assumption and show that the model has no basis in reality.

It is interesting to reflect that if the determiners of heredity had chanced to be of a lower order of magnitude,

below the level at which the experimental study of individual cases is possible, we might still be constructing more and more refined models of the gene on this pattern. As the predictions made from the model were contradicted by experimental results, we would change the various numerical values, or introduce additional variables, or perhaps, if necessary, even create additional hypothetical units. But the model would remain essentially an imaginary construct inferred from mere numbers of mutations, for we would have no possibility of contradicting the plausible assumption that one mutation is as good as another.

WHAT IS A GENE?

The early studies of gene mutation were concerned mainly with problems of technique arising from the extreme rarity of the phenomenon. Although the mutations of *Oenothera*, on which the mutation theory was based, had proved illusory, it soon became evident that mutant alterations do occur that are inherited as if they were due to changes in individual genes. The comprehensive genetic analysis of *Drosophila* by Morgan and his coworkers showed numerous cases of this sort—in fact, almost all the loci shown on the gene-map represented the mutant occurrence of visible alterations which, on subsequent tests, proved to be inherited in typical Mendelian fashion. These were assumed to be due, in each case, to a change of the wild-type gene to an alternative form, producing a recognizably different phenotypic effect. The frequency of these mutations, however, seemed far too low to permit experimental investigation of the conditions affecting their occurrence.

Muller (4) pointed out in 1917 that gene mutations resulting in inviability

("lethals") are probably more frequent than mutations permitting survival with modified phenotype ("visibles"). In experiments extending over the next 10 years (5), he developed various special techniques by which it was possible to determine the total number of lethal mutations for all loci within a given chromosome or region. These total frequencies proved to be high enough to permit significant experimental comparison of mutation frequencies under different temperatures. The loci yielding lethal mutations were distributed over the chromosomes approximately as expected from the distribution of loci for visible mutants, and it was concluded that the lethal mutations might legitimately be used as an index of gene mutations in general.

Meanwhile, many attempts to increase the frequency of genetic alterations by external treatments had been made, including studies with various chemical, radiological, and serological treatments, and studies in which various plant and animal forms were used. None of these experiments gave conclusive proof of an effect of any experimental treatment on the frequency of mutation, although in several of the experiments there were genetic alterations that may have been induced by the treatment. The failure of proof was due to two difficulties: (i) that of proving that the genetic alterations observed in the progeny of treated individuals were in fact due to the treatment rather than to some genetic irregularity present in the treated strains, and (ii) that of showing statistically convincing increases in the frequency of mutations in the treated group. What was needed was a genetic technique suitable for the detection of mutations in adequate numbers in an organism in which the gene-determined inheritance of the mutant char-

actors could be readily demonstrated.

The "C1B" technique with *Drosophila*, designed by Muller, was admirably suited to this purpose, and x-ray experiments with this technique (6, 7) demonstrated beyond question a very strong effect of x-rays on the frequency of mutation. The total frequency of lethals in the X-chromosome was increased more than 100-fold. In addition, many visible mutations were found, including dominants as well as recessives, and including mutants previously known from their spontaneous occurrence as well as many mutants not previously observed.

These experiments were promptly followed by others designed to test more critically the genic nature of the induced mutations. The mutant lethals might be suspected of being deficiencies; even the visibles could conceivably be due to short deficiency or gene destruction. But if the treatment could induce mutation to a variant allele and could, in further applications, induce reverse mutation to the parental allele, it was argued, the two mutations could not both be due to gene loss. Induced mutation and induced reverse mutation at the same locus were shown to occur in a number of loci of *Drosophila* in experiments by Patterson and Muller (8) and Timoféeff-Ressovsky (9).

Subsequent experiments with a wide variety of forms among the higher plants and animals and with micro-organisms showed the broad generality of the effects of ionizing radiations upon the frequency of mutation. In later experiments, ultraviolet radiation and various chemical treatments were also shown to affect mutation frequency.

The analysis of the induced mutations, however, soon indicated that the

accepted definitions and criterions related to genes and gene mutations needed reconsideration.

The purpose of experiments with gene mutation is to study the evolution of new gene forms. The techniques for studying gene mutation are, therefore, designed to measure the frequency of these changes in the genes. But a change in the gene may be recognized only by its effects, and it soon became clear that various extragenic alterations might produce the effects considered characteristic of typical gene mutation (10).

Thus the working definition of mutation necessarily differs from the ideal definition. It is this working definition that must be considered in generalizing from the experimental evidence. The mutations experimentally identified as gene mutations may include not only variations due to alterations within the gene but also variations due to losses of genes, to additions of genes, and to changes in the spatial relationships of genes to one another. To identify these mechanical alterations, certain tests were applicable. But there was no test to identify mutations due to a change within the gene; it was simply inferred that the mutants that could not be identified as the result of specific mechanical causes were, in fact, due to gene mutation in the ideal sense (11).

When we conclude from an experiment that new genes have been evolved by the action of x-rays, we are not simply stating the results of the experiment. We are, in the single statement, combining two distinct steps: (i) stating the observed results of the experiment, and (ii) interpreting the mutations as due to a specific mechanism. It is essential that these two steps be kept separate, because the first step represents a permanent addition to the known body of fact, whereas the sec-

ond step represents only an inference that may later be modified or contradicted by additional facts. When the two steps are unconsciously combined, we risk confusing what we know with what we only think we know.

The widely held belief that the frequency of gene mutation may be greatly accelerated by x-ray treatment was an illusion of this kind. Its basis was the use of the term *gene mutation* with two distinctly different meanings. Gene mutation was thought of as a change in the constitution of a unit of the genetic material, producing a new gene with altered gene action. Gene mutation was identified in experiments by the occurrence of a mutant character inherited as if it were due to a change in a gene.

The mischief involved in the use of the same term for the two concepts is obvious. To insist that x-rays induce gene mutation because the mutants induced satisfy all the accepted criterions of gene mutation, and that these mutants represent qualitative changes in specific genes because that is what we mean by gene mutation, is to adopt the dictum of Humpty Dumpty in *Through the Looking-Glass*. "When I use a word," Humpty Dumpty said, "it means just what I choose it to mean—neither more nor less."

Now our concept of the gene is entirely dependent upon the occurrence of gene mutations. If there were no gene mutations, we could not identify individual genes, because the total genetic effect of a single chromosome would be inherited as a unit. If the mutations we interpret as gene mutations are in fact due to alterations affecting groups of genes, then the entities that we will recognize as genes will be in fact the corresponding groups of genes. The significant ambiguity is not in our definition of gene

mutation but in our definition of the gene itself, because any definition of gene mutation presupposes a definition of the gene.

The discussion of these difficulties and of the possibility of remedying them by more rigorous definition of experimental concepts is only an application to biology of the operational viewpoint that has become commonplace in modern physics, largely as a result of the critical studies of P. W. Bridgman (12). As Bridgman notes, this sort of critical reconsideration, made necessary in physics by the development of relativity, is essential in scientific thinking if the methods are to be made elastic enough to deal with any sort of facts that may develop. The essential feature of the operational viewpoint is that an object or phenomenon under experimental investigation cannot usefully be defined in terms of assumed properties beyond experimental determination but rather must be defined in terms of the actual operations that may be applied in dealing with it. The principle is not a new one; it has been recognized, at least implicitly, in the work of individual scientists from an early period. William James stated it essentially in his lectures on pragmatism (13), illustrating it with a quotation from Wilhelm Ostwald:

Chemists have long wrangled over the inner construction of certain bodies called tautomerous. Their properties seemed equally consistent with the notion that an unstable hydrogen atom oscillates inside of them, or that they are unstable mixtures of two bodies. Controversy raged but never was decided. "It would never have begun," says Ostwald, "if the combatants had asked themselves what particular experimental fact could have been made different by one or the other view being correct. For it would then

have appeared that no difference of fact could possibly ensue; and the quarrel was as unreal as if, theorizing in primitive times about the raising of dough by yeast, one party should have invoked a 'brownie' while another insisted on an 'elf' as the true cause of the phenomenon."

What is a gene in operational terms? In other words, how can we define the gene in such a way as to separate established fact from inference and interpretation? The definition may take into account not merely the evidence from experiments on the occurrence of mutations but also the evidence from experiments on the inheritance of genetic differences of any kind, or from any other experiments that bear on the nature of the gene. The definition may specify attributes of the gene that can be determined by recognized experimental operations, whether these are attributes already established in past experiments or attributes that might be determined in future experiments.

Operationally, the gene can be defined only as the smallest segment of the gene-string that can be shown to be consistently associated with the occurrence of a specific genetic effect. It cannot be defined as a single molecule, because we have no experimental operations that can be applied in actual cases to determine whether or not a given gene is a single molecule. It cannot be defined as an indivisible unit, because, although our definition provides that we will recognize as separate genes any determiners actually separated by crossing over or translocation, there is no experimental operation that can prove that further separation is impossible. For similar reasons, it cannot be defined as the unit of reproduction or the unit of action of the gene-string, nor can it be shown to be delimited from neighboring genes by definite boundaries.

This does not mean that questions concerning the undetermined prop-

erties mentioned are meaningless questions. On the contrary, they are the all-important questions that we hope ultimately to answer by the interpretation of the experimental evidence and by the development of new experimental operations. The operational definition merely represents the properties of the actual gene, so far as they may be established from experimental evidence by present methods. The inferences from this evidence provide a tentative model of the hypothetical gene, a model that will be somewhat different in the minds of different students of the problem and will be further modified in the light of further investigation.

The term *gene* as used in current genetic literature means sometimes the operational gene and sometimes the hypothetical gene, and sometimes, it must be confessed, a curious conglomeration of the two. The resulting confusion may be strikingly illustrated in seemingly contradictory statements by two such gifted and clear-sighted geneticists as Richard Goldschmidt and A. H. Sturtevant. Goldschmidt, after reviewing the evidence on position effect, states that genes do not exist (14), or at any rate that the classical theory of the corpuscular gene must be discarded (15). Sturtevant, citing the evidence that chromosomes are regionally differentiated, that particular regions are necessary for particular reactions in the organism, and that these particular regions behave as units in crossing over, states "These propositions . . . prove the existence of genes" (16).

Goldschmidt is essentially correct if, by the gene, we mean the hypothetical gene, and the particular hypothetical gene that he has in mind. His positive conclusion that the gene does not exist is prone to misinterpretation but apparently means only that this hypothetical gene does not exist. His con-

tention that the properties commonly ascribed to "the classical, corpuscular gene" go far beyond the evidence is, I think, fully justified.

Sturtevant is correct if, by the gene, we mean the gene of the operational definition, since this implies no unproved properties. If it were true that there are no discrete units in the gene-string, Sturtevant points out, the most direct way of establishing the fact experimentally would still be by studying the properties and interrelationships of these distinguishable regions. These are the genes of the operational definition.

What is the operational definition of gene mutation? We have recognized that our studies of gene mutation have significance for the major problem only to the extent that they identify and analyze the mutations that represent the evolution of new hereditary units. But it is obvious that no operational definition of gene mutation in this sense can now be formulated—for these hereditary units are not the genes of the operational definition; they are the hypothetical genes postulated in our interpretation of the experimental evidence. To say that no operational definition is now possible is only to repeat in different words the foregoing statement that we have no positive criterion to identify mutations caused by a change within the gene, and that the alterations interpreted as gene mutations in experiments are merely the unclassified residue that cannot be proved to be due to other causes. The major objective in further investigations must be to develop such criterions.

STUDY OF THE MUTATION OF SPECIFIC GENES

The main purpose of this paper (17) is to emphasize the unpleasant fact that significant progress in our understanding of gene mutation requires the

investigation of the mutation of specific genes. The fact is unpleasant because the various technical difficulties that arise from the very low frequency characteristic of mutation are at their worst when the study must be made on single genes, particularly on the spontaneous mutation of single genes. The unpleasant statement is a fact because, as we have seen, it is hopeless to identify and exclude the spurious or extragenic mutations in experiments on mutation rates at miscellaneous unspecified loci.

The chief advantage in focusing the study on the single gene is that this makes it possible to substitute the direct experimental analysis of specific mutants for the application of generalizations assumed to apply to mutations at all loci. Each mutant studied may add to the background of detailed information available for the diagnosis of other mutants of the same gene.

An important further advantage is that the specific loci selected for study may be loci with unusual technical advantages for the recognition and analysis of their mutants. For example, the genes R^r and A^b in maize, like other known genes in various species, yield spontaneous mutants that are clearly distinct from the forms produced by recognizable short deficiencies at these loci. This does not prove that the spontaneous mutants are not due to still smaller deficiencies, but it supplies a convenient screen for identifying a large class of deficiencies without further investigation. Another very useful aid in discriminating between gene loss and gene alteration is available for the recessive allele a . This allele, although phenotypically distinguishable only by the loss of A action, may be distinguished from gene deficiency by its response to the mutagenic gene Dotted (Dt), in the presence of which it reverts sporadically to the dominant allele A . The retention

of the *Dt* response provides a criterion to exclude gene loss in the interpretation of experiments on spontaneous and induced mutation of *A*. A technical advantage of a different sort is provided by the *R* alleles. The phenotypic effect of *R* is such that a large number of alleles may be objectively distinguished by very slight differences of plant color intensity and pattern. A gene with equally variable allelic forms, if identified only by its effect on some all-or-none response, would seem to have only two alleles, and its mutations would not be detectable except for those that crossed the line between these two distinguishable levels of action. Another advantage of great practical importance is that both *R* and *A* are genes affecting endosperm characters and are, therefore, suitable for the identification of mutations in large populations. Both are apparently genes of such trivial effect physiologically that their mutants survive with no detectable loss of viability.

The effective analysis of the diverse genetic phenomena that may result in the origin of a Mendelizing variation may not be impossible in intensive studies of the mutations of suitable selected genes, despite the fact that it seems hopeless in studies of mutation at miscellaneous, unspecified loci.

These considerations are of no account if the frequency of spontaneous mutation of the single gene is actually too low to permit effective experimental study. We cannot safely avoid this difficulty by selecting for study the genes of unusually high mutation frequency, because there is no assurance that the mechanism responsible for the behavior of "unstable genes" is representative of the mechanisms concerned in typical gene mutation. The use of microorganisms that permit effective screening for mutants in virtu-

ally unlimited populations would remove the difficulty, but unfortunately these do not provide the critical genetic background essential to the study.

A technique for determining the spontaneous frequency of mutation of specific genes is practicable in maize for mutation rates ranging as low as about one per 1 million gametes (18). A test of eight genes, unselected except for the technical advantage of showing their effects in the endosperm, yielded mutations in all but one of the genes tested, the mutation frequencies ranging from about one to about 500 per 1 million gametes tested (19). The genes that yielded mutations in sufficient numbers to permit the comparisons showed rather wide variation in mutation frequency in different cultures. The gene *R*, for example, yielded no mutations in large populations in some cultures, but its mutation rate in other cultures ranged as high as 0.2 percent. Later studies have shown that such differences are due in part to differences intrinsic to the *R* allele concerned and in part to differences caused by factors modifying the mutation rate of *R* (20). Such factors are apparently quite common, since a study in which only strong effects could be detected indicated the occurrence of such modifiers in three of the seven regions marked (21).

The average mutation rates determined are rather low for effective experimental investigation of factors affecting the mutation rate and even for the extraction of adequate samples of mutants for individual study. However, the fact that mutation rates are so readily affected by diverse modifiers makes it feasible to extract strains in which the mutations of specific genes may be made frequent enough to permit direct experimental study.

DETECTION OF SPURIOUS GENE MUTATIONS

The development of criterions for identifying gene mutations of evolutionary significance is difficult even in the study of selected genes of the most favorable properties. In past studies, the problem has been given a disarmingly simple appearance by various assumptions, some of which were unwarranted, and some of which have been invalidated by later discoveries.

For example, we tend to feel that some of the mutations detected in our experiments must be qualitative changes in the genes concerned, for surely qualitatively altered genes have arisen in the course of evolution. This is mainly responsible for the widespread belief that, even though some of the apparent gene mutations identified are demonstrably false, "true" gene mutations must be included in the unclassified residue.

This belief is fallacious. Granting that qualitatively changed genes must have been evolved by mutation at rates high enough to permit experimental investigation, there is no assurance that the steps in their evolution are represented in the mutants that are found in our mutation experiments. When we set out to identify mutants in a mutation experiment, we must confine ourselves to mutations of relatively large effect, large enough to set the mutant beyond the range of varying expression due to environmental and genetic modifiers. If mutant changes occur within the narrower range, we have no way of identifying them. There is no good evidence against the occurrence of such subliminal mutations. The assumption of the high constancy of the gene is backed by evidence only concerning the rarity of the distinct mutations. If convincing evidence were

adduced tomorrow to show that genotypes breed true only as a statistical result of sampling in each generation in populations of genes genetically fluctuating over an imperceptible range, there is nothing in our present knowledge that would contradict this conclusion.

A study of *R* alleles of diverse origin showed the common occurrence of minute differences in the level of plant-color expression (22). Such allelic differences would not be expected if the only source of variation in this gene were mutation of the type that we study in our experiments, but they would be expected as a result of subliminal mutation.

If subliminal mutations occur, it is possible that this type of mutation accounts largely or wholly for the evolution of new gene forms in nature. Thus it is quite possible that the sharply distinct mutations identified in our experiments may be exclusively the result of extragenic phenomena.

A second assumption, or group of assumptions, is concerned with the possibility of distinguishing gene mutation from gene loss. It was originally supposed that induced recessive "visibles" could safely be considered gene mutations, on the assumption that all genes were essential to survival. This was contradicted by various instances of cytologically demonstrable deficiencies viable in haploid tissue or in hemizygous individuals, or viable as homozygotes in diploid individuals. Such cases were relatively few, but since both the cytological and the genetic criterions of deficiency approach the limit of their range of effective application as the deficient segment becomes smaller, there is reason to suspect that physical loss may be responsible for observed mutations also in cases in which deficiency can-

not be demonstrated. As we have become better acquainted with individual genes and their functions, the assumption that genes, as a rule, are individually essential to life has lost its plausibility.

Mutation to an intermediate allele is sometimes considered evidence against loss mutation. This involves another assumption, that of the unitary nature of the gene—an assumption made consciously and with careful consideration in the early development of gene theory, but one that must be seriously questioned in the light of later evidence. It is only on the hypothesis that multiple alleles are variant forms of a single unit that we may exclude the possibility of their occurrence by loss mutation. On the hypothesis that they represent different mutations in a complex of closely linked genes, we could account for mutation to different levels by the loss of different segments of the chain.

The basis for the choice of the unitary hypothesis is perhaps best shown in the considerations underlying the classical criterion of allelism. These were stated by Morgan in 1919 (23) as follows:

Probably the most important evidence bearing on the nature of the genes is that derived from multiple allelomorphs. Now that proof has been furnished that the phenomena connected with these cases are not due to nests of closely linked genes, we can probably appeal to these as crucial cases. . . . The demonstration that multiple allelomorphs are modifications of the same locus in the chromosome, rather than cases of closely linked genes, can come only where their origin is known. . . .

A
1 ●
2 ●
3 ●
4 ●
5 ●

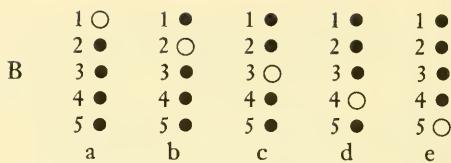


Fig. 108 [in part]. Diagram illustrating mutation in a nest of genes so closely linked that no crossing over takes place.

Let the five circles of Fig. 108, A represent a *nest* of closely linked genes. If a recessive mutation occurs in the first one (line B, a) and another in the second gene (line B, b), the two mutants *a* and *b* if crossed should give the atavistic type, since *a* brings in the normal allelomorph (*B*) of *b*, and *b* that (*A*) of *a*. . . . Now this is exactly what does *not* take place when members of an allelomorphic series are crossed—they do not give the wild type, but one of the other mutant types or an intermediate character. Evidently independent mutation in a nest of linked normal genes will not explain the results if the new genes arise directly each from a different normal allelomorph.

It will be noted that the test rules out the existence of the nest of closely linked genes only on the assumption that each mutation must be an alteration of a single number of the group. If, instead, each mutation were a loss of one or more contiguous numbers of the group, the fact that crosses between them might commonly show them to be allelic would not rule out the "compound gene" as the basis of the multiple allelic series. This is illustrated in the following diagrammatic arrangement:

1 ●	1 ●	1 —	1 ●	1 ●
2 —	2 ●	2 —	2 ●	2 ●
3 —	3 —	3 —	3 —	3 —
4 ●	4 —	4 —	4 ●	4 —
5 ●	5 —	5 ●	5 ●	5 —

The "compound gene" is in a sense a contradiction in terms, for the hypo-

theoretical gene is unitary by definition. But the genes identified in our experiments cannot be made unitary by definition. The five genic elements represented in the diagram are not actually parts of one gene; they are five genes. But if certain multiple allelic series have a basis of this type, it would be possible to establish the fact experimentally only in the cases most favorable for analysis. Accordingly, there might be many cases in which the segment of the gene-string identified experimentally as a single gene might actually be a cluster of genes of identical or similar effect.

The notion of the compound gene, or some equivalent unit, may prove to have significance, since there may be special relationships among the clustered elements that mark them off as a group from adjoining unrelated elements. One of these may be interrelationships in gene action between the clustered elements, which could lead to the occurrence of position effects when members of the cluster are separated by crossing over or translocation. This may be a basic factor in the explanation of position effect in general. Another relationship to be expected is synaptic equivalence, leading to the opportunity of unequal crossing over. It is the latter that concerns us here.

A striking example of minute deficiencies simulating gene mutations is provided by the "crossover-mutants" of R^r (24). Certain R^r alleles consist of at least two independently mutating genic elements: (P), determining anthocyanin pigmentation of certain plant tissues and of the pericarp, and (S), determining anthocyanin pigmentation of the endosperm and embryo. The crossover-mutants R^g and r^r result from unequal crossing over and must, therefore, involve the loss of (P) in the one case and of (S) in the

other. They give no cytological or genetic indication of deficiency, and they are wholly normal in development in the haploid gametophyte, as is shown even by the very sensitive test of competitive pollen-tube growth in the transmission of the mutant through male germ cells. The cross-over-mutants are wholly indistinguishable in appearance and genetic behavior from the noncrossover mutants occurring in the same cultures.

The occurrence of unequal crossing over within the R complex yields some interesting indications of the genetic nature of multiple allelic series and of the possible role of gene losses in relation to seemingly qualitative mutations. In addition to (P) and (S), there are other phenotypically recognizable genic elements of the R complex. In certain R^r alleles of dilute pigmentation, both plant and seed color are dependent upon a single genic element (D). In various R^r alleles of unusually strong pigmentation, there appear to be additional elements determining certain aspects of plant-color expression. In addition, there are various distinguishable aleurone-color types such as "Stippled," "Marbled," "Navajo-spot," and so forth, some occurring with plant color and some without. Each of the distinguishable complexes may be regarded as one of a long series of multiple alleles of the gene R .

Let us pause a moment to clear the terminology. To avoid confusion I shall refer to the recognized alleles of R under their customary italicized designations (R^r , R^g , r^r , R^{Nj} , and so forth), although the analysis shows that several of these so-called "alleles" are actually complexes of two or more genes.

The term *genic element* will be used for any gene-like constituent identified as a component of one of the R alleles. The use of this term does not,

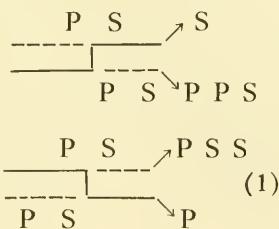
in the absence of further evidence, necessarily imply that the element is unitary. The genic elements are designated by symbols not italicized, such as P, S, D, and so forth.

In addition to the crossover mutants there are numerous noncrossover mutants. A noncrossover r^r mutant is presumably of constitution "P s" rather than merely "P." The postulated element (s) is a "null" element phenotypically but presumably would function synaptically in the same way as "S." These postulated elements are designated "s," "p," "d," and so forth.

The complex may, of course, include other null elements from past mutations in which the parental elements are unknown. These as a class are designated as "n."

In several instances noncrossover mutants to intermediate levels of seed-color expression occurred including various dilution and pattern types. These are designated "S^d," "S^s," and so forth.

Once any two of these genic elements have become established in neighboring positions in the same chromosome, an opportunity is provided for unequal crossing over, which may ultimately lead to the development of more complex gene clusters. For example, the aforementioned crossover mutants resulted from interchanges as follows:



The crossover-product "S" was recognizable as a crossover mutant R^g and the crossover-product "P" as crossover mutant r^r . The crossover-prod-

ucts "P P S" and "P S S" were not recognizable, but these represented the production of potential new alleles carrying three genic elements instead of two. By using distinguishable forms of S or P in the original compound, the addition-crossovers may be made recognizable, and by this means it is possible to produce such new synthetic alleles as R (Stippled-Navajo), and so forth. In this manner, it would be expected that more complex clusters would develop by successive steps, unless the gene is one whose action sets a closer limit on the viability of its duplications.

The great variety of genotypes that might be expected to represent possible members of the allelic series may be illustrated by a few examples as follows:

- 1) S S p n
- 2) S P P n S
- 3) D
- 4) D S P
- 5) S^s P D

Alleles (2) and (4) would be of the standard R^r type, (3) would be of the dilute R^r type, (1) would be of the R^g type, and (5) would be a spotted aleurone type with plant color. In general, the differences between the alleles are due to extragenic, rather than intragenic, alterations, but this is not necessarily true of the phenotypic difference between (4) and (5).

With regard to the relationships between the genic elements of the complex, the concepts of allelism and locus have little meaning. All members of the complex are homologous with one another; presumably all have arisen through a long series of mutations from some single ancestral gene. In a sense, all may be considered allelic to one another. For example, the question "Is Sⁿ (the seed-color element in R^{Nj})

allele to S?" has no significance, because there is no way in which S^n can be shown to have any different relationship to S than to P or to any other element of the complex. The same is true of such a question as "Is the element (D) proximal or distal to (P)?" It may be proximal in one stock and distal in another; in a stock in which it is proximal, a short series of unequal crossovers will suffice to move it to a distal position.

Although different alleles may have widely different numbers of genic elements, none is actually a deficiency. In terms of the postulated origin of the cluster, all of those with more than a single element may be considered duplications. On the other hand, when we arbitrarily take as the standard type an allele carrying several genic elements, other alleles with fewer elements will appear as deficiencies, and the mechanisms that produce them as mutants from the standard type will be mechanisms of gene loss.

The same mechanisms proceeding in the case of a gene-complex whose separable elements are identical in action might produce only a linear series of multiple alleles showing various grades of dilution, or they might produce no multiple series of alleles at all.

The increasing number of cases in which clustering of genes of identical or similar effect is proved or indicated (24-27 and others, 28 and 29 for references) suggests that unequal crossing over may be a significant factor in the production of seemingly qualitative allelic differences.

Another simplifying assumption was that mutant changes in gene effect must represent some transformation of the gene itself rather than some alteration affecting its expression. It was this assumption that made the demonstration of x-ray-induced mutation and reversion of the same gene seem

critical proof of the induction of intragenic alterations. The assumption was definitely contradicted by the evidence of position effect. This evidence showed conclusively that a mutation did not necessarily represent a transformation or loss of the gene concerned; instead, it could be the result of a translocation affecting the expression of the unchanged gene.

The remarkable studies of McClintock (30, 31) on mutational behavior in maize, as affected by the introduction of a chromosome-9 undergoing the breakage-fusion-bridge cycle, have shown the far-reaching importance of this limitation in the experimental study of gene mutation. In the presence of this structurally unstable chromosome, many of the type genes present, including genes in chromosome-9 and genes in other chromosomes, show mutation to unstable recessive forms characterized by various types of chromosomal irregularity. The study of the unstable mutants and their reversion leaves little doubt that the phenomenon is due to some reversible inhibition of the expression of the genes concerned.

In some cases the mutations are accompanied by detectable chromosomal aberrations at or near the locus showing instability, but in other cases no cytologically detectable chromosomal alteration is associated with the occurrence of the mutation. In many cases the instability of the recessive mutant and the occurrence of the associated chromosomal irregularities are dependent upon the presence of a complementary factor designated "activator" (Ac), and when this factor is removed the mutant behaves as a stable recessive with normal chromosomal behavior.

McClintock has also shown that the control of reverse mutation of the recessive *a* by *Dt* (Dotted) may be a re-

action of the activator type. In the presence of the aberrant chromosome-9 and in the absence of *Dt*, the standard *a* allele has given occasional endosperm dots apparently due to mutation to *A*. This strongly indicates that the standard *a* is a repressed *A*, and, if so, its reversion under the influence of *Dt* must also be due to some modification of conditions affecting gene expression.

Whether or not there is acceptance of my hypothesis that these manifestations of unstable gene behavior are brought about by the transposition of invisible bits of heterochromatin to the locus of the gene affected, this brilliant investigation clearly shows that expression effects may be the actual cause of apparent gene mutations, even when the mutation observed shows no indication of a change of position or of any associated chromosomal alteration.

The resulting difficulty in the analysis of observed mutations further emphasizes the necessity for carrying on the analysis with the advantages of the detailed study of mutation at specific loci. If we think of these results in terms of the generalizing assumptions characteristic of the study of mutation *en masse*, we may be inclined to apply the findings to the nature of gene instability in general, or even to the nature of mutant alleles in general. If we think of them against the background of diverse mutations of some intensively studied gene, we are inclined to make detailed comparisons of the mutants of this category with those of other types and other modes of origin in the hope of developing criterions that distinguish mutants of different kinds.

Meanwhile, in the study of gene mutation, we are for the present in an anomalous position. A mutant may meet every test of gene mutation, and yet, if it is not capable of reverse

mutation there is ground for the suspicion that it may be due to gene loss, while, if it is capable of reverse mutation, there is ground for the suspicion that it may be due to an expression-effect. The only escape from this dilemma is through the more intensive study of the mutations of specific genes selected as best suited to detailed genetic analysis, in the hope of developing more sensitive criterions for the identification of gene mutations.

REFERENCES AND NOTES

1. Muller, H. J., *Am. Naturalist* 56:32, 1922.
2. Delbrueck, M., *Nachr. Ges. Wiss. Gottingen* (Math. physik. Kl., Biol.) 1:223, 1935.
3. Schrödinger, E., *What is Life?* (Cambridge Univ. Press, New York, 1944).
4. Muller, H. J., *Proc. Natl. Acad. Sci., U. S.* 3:619, 1917.
5. ———, *Genetics* 13:279, 1928.
6. ———, *Science* 66:84, 1927.
7. ———, *Z. ind.*, Suppl. 1:234, 1928.
8. Patterson, J. T., and Muller, H. J., *Genetics* 15:495, 1930.
9. Timoféeff-Ressovsky, N. W., *Naturwiss.* 18:434, 1930.
10. Stadler, L. J., *Sci. Agr.* 11:645, 1931.
11. ———, *Proc. 6th Intern. Congr. Genet.* 1:274, 1932.
12. Bridgman, P. W., *The Logic of Modern Physics* (Macmillan, New York, 1927).
13. James, W., *Pragmatism* (Longmans, Green, London, 1907).
14. Goldschmidt, R., *Sci. Monthly* 46:268, 1938.
15. ———, *Experientia* 2:1, 1946.
16. Sturtevant, A. H., in *Genetics in the 20th Century* (Macmillan, New York, 1950), pp. 101-110.
17. Given as the presidential address, American Society of Naturalists, annual meeting, Boston, Mass., Dec. 30, 1953. It is a report of the cooperative investigations of the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and Department of Field Crops, University of Missouri (*Missouri Agri. Expt. Sta. J.*, Ser. No. 1409). The work was aided by a grant from the U. S. Atomic Energy Commission.

18. Stadler, L. J., *Genetics* 31:377, 1946.
19. ———, *Spragg Memorial Lectures*, 3rd ser. (Michigan State College, East Lansing, 1942), pp. 3-15.
20. ———, *Am. Naturalist* 82:289, 1948.
21. ———, *ibid.* 83:5, 1949.
22. ———, *Cold Spring Harbor Symposia* 16:49, 1951.
23. Morgan, T. H., *The Physical Basis of Heredity* (Lippincott, Philadelphia, 1919).
24. Lewis, E. B., *Genetics* 30:137, 1945.
25. Green, M. M., and Green, K. C., *Proc. Natl. Acad. Sci., U. S.* 35:586, 1949.
26. Laughnan, J., *ibid.* 35:167, 1949.
27. Silow, R. A., and Yu, C. P., *J. Genet.* 43:249, 1942.
28. Green, M. M., *Proc. Natl. Acad. Sci., U. S.* 40:92, 1954.
29. Stephens, S. G., *Advances in Genetics* 4:247, 1951.
30. McClintock, B., *Proc. Natl. Acad. Sci., U. S.* 36:344, 1950.
31. ———, *Cold Spring Harbor Symposia* 16:13, 1951.



Social Implications of the Genetics of Man*

A. H. STURTEVANT

Reprinted by author's and publisher's permission from *Science*, vol. 120, 1954, pp. 405-407.

As I mentioned above (p. 194), this paper and the report of the Genetics Conference point a new direction for genetic thought. The Genetics Conference paper indicates a new trend in methods as well, that of team research.

This is not to say that the day of the individual is over. As this paper demonstrates, thought is still the prerogative of a single mind. Intelligent analysis of a problem will always depend upon an individual's experience, abilities, accumulated knowledge, and logical reasoning. It is good to have men like Sturtevant, Linus Pauling, and Edward Teller concerning themselves with the impact of genetics on society. Whether they be right or wrong, their arguments and discussions force us all to think a little more clearly about the problems we face as human beings.

MAN IS ONE OF THE MOST UNSATISFACTORY of all organisms for genetic study. The time interval between suc-

cessive generations is long, at best individual families are too small to establish ratios within them, and the test-matings that a geneticist might want cannot be made. Obviously no geneticist would study such a refractory object, were it not for the impor-

* Presidential address at the Pacific Division of A.A.A.S., Pullman, Wash., June 22, 1954.

tance that a knowledge of the subject has in other fields.

One consequence of the difficulty of the material is that the exact mode of inheritance is known for very few of the differences among individuals. It is important that suspected cases be recorded, in order that other workers may check them; but there is an unfortunate tendency to accept such records as demonstrations rather than as suggestions. After examining some of the available published evidence, I am convinced that, even for some of the standard textbook examples, the evidence for the accepted mode of inheritance is far from conclusive—and that it would be recognized as at best suggestive, if any organism other than man were concerned.

There are enough unambiguous examples known to make it clear that the same principles are at work in man as in all other higher animals and plants—and even without such evidence, enough is known about the cytology of human tissues to give us confidence that no peculiar kind of inheritance is to be expected in man. In fact, much of the argument concerning the practical aspects of the genetics of man is best based on experimental evidence from other organisms rather than on what is known directly from study of human populations.

The position is especially unsatisfactory with respect to the heritability of the most important of all human differences—namely, mental ones. It would be possible to quote recent authorities for rather extreme positions on each side of this question. To some there appears to be no clear evidence for genetic differences in mental capacities among most individuals or among races, the observed mental diversity being attributed to environmental effects; to others the position is reversed—the environment accounts

for little, genetic differences for nearly all the observed diversity. In these circumstances it is necessary to examine what direct evidence we have.

At the sensory level there is good evidence for inherited differences. There can be no question that such things as color-blindness, night-blindness, or sensitivity to the bitter taste of phenylthiourea are simply inherited; and one may confidently suppose that other such inherited sensory differences remain to be discovered. As has been pointed out by Blakeslee, we all live in different worlds by virtue of inherited differences in our sensory reactions to external stimuli. It should further be pointed out that these differences have effects at the highest mental levels. About 8 percent of white males are at least partially red-green blind; and when such a man looks at a painting he does not see what the artist put there or what other people see. It is clear that this simple and rather frequent genetic property has inevitable effects on the esthetic life of the individual.

These remain rather trivial sorts of differences; but there is another large class of inherited mental differences that is far from trivial. Certain types of severe mental derangement, such as Huntington's chorea or phenylketonuria, clearly have at least a large inherited element in their causation, although for most of them the exact method of inheritance may be regarded as somewhat uncertain.

However, what we are really most interested in is the vast array of differences lying between these extremes; and it is just here that the difficulty of the human material becomes most serious. When one is dealing with complex characters that vary more or less continuously in diverse respects, a genetic analysis is difficult in any material; in the case of man, a direct attack on the

problem looks even more difficult.

One thing we want to know is: What portion of existing mental diversities is of genetic origin and what portion is of environmental origin? Under these conditions the usual scientific procedure is to try to hold one variable constant, and then study the effects of variations in the other one. This can in fact be approximated in the problem of human mental differences, through the study of twins. Ordinary fraternal twins arise from the separate fertilization of two eggs and are no more alike genetically than are brothers and sisters that are not twins; but the environment to which they are subjected is likely to be more nearly the same. Identical twins arise from a single fertilized egg and are genetically identical. If one studies members of such pairs that were separated in infancy, any observed differences must be nongenetic.

The difficulty here is in the measurement of the properties we are interested in. Such studies of separated identical twins were begun by Muller and have been greatly expanded by Newman, Freeman, and Holzinger. I must confess to a feeling, however, that these studies tell us more about what the psychological tests used are really measuring than they do about the relative effects of heredity and environment.

There are then inherited differences in the sensory components of human mentality and also in components leading to severe derangements. In the area between these extremes the technical difficulties of getting clear-cut evidence are still unsurmounted. But it seems safe to conclude, from what we know of the genetics of complex characteristics in other organisms, that any property as complex and as variable as this must have a large amount of underlying genetic diversity.

This conclusion applies to individual differences. Analogy with other organisms leads likewise to the conclusion that there must also be at least statistical differences between racial groups. This is a rule that has held consistently wherever it has been tested—in many different kinds of animals and plants.

On general grounds, then, as well as from some direct evidence, one must conclude that there are inherited differences in mental properties among individuals and, at least statistically, among racial groups. But it is necessary to insist that one must not go beyond this point. Specifically, one must not conclude that a particular observed difference is genetically determined. It is, of course, a platitude to say that no one ever does anything for which he does not have the necessary genes; but one must never forget that there is also a necessary environment. It scarcely needs argument that human behavior is strongly influenced by economic status, tradition, and training. After all, most of the members of this society are in the business of teaching or, at least, have spent a good deal of time and energy pursuing academic work; we are therefore all of us witnesses to the obvious fact that men are teachable—that their behavior can be strongly modified by environmental stimuli.

This caution about attributing observed differences to genetic causes, rather than to environmental ones, applies with special force to comparisons among racial groups, for here the effects of tradition and of public opinion are especially strong.

Another thing that must be avoided is the view that one race (usually that to which one himself belongs) is "better" than another. All that can properly be concluded is that they are inherently different. It follows that

society would do well to insure that as many people as possible, of as diverse racial origins as possible, get an opportunity to show what they can do to advance civilization. It may confidently be expected that individuals of various races will have the necessary genetic equipment to make unique contributions.

I wish to devote the rest of this paper to the effects of high-energy radiation on the genetic properties of man. This is a matter that has been of significance chiefly because of the use of x-rays for diagnostic and therapeutic purposes; but with the development of A-bombs and H-bombs it has become of far more general importance, for it is already true that all of us have been subjected to irradiation from these sources.

There are two possible types of radiation damage to be considered—damage to the exposed individual, and damage to the genes in his germ cells. The first will be more or less immediate in its manifestation, whereas the latter will have detectable effects only in future generations. This, however, is not the most basic distinction. Irradiation has a gross effect on tissues, resulting in the burns and other symptoms recognized as direct effects of heavy dosages; there is also an effect on the genes, leading to mutations.

The former, tissue effect, appears to be substantially absent at low doses, recovery from moderate effects is possible, and doses spaced well apart in time have little or no cumulative effects. It is on the basis of these effects that the "permissible" dose, to which it is supposedly safe to expose individuals, is calculated. But there is reason to suppose that gene mutations, induced in an exposed individual, also constitute a hazard to that individual—especially in an increase in the probability of the development of malign-

nant growths, perhaps years after the exposure. There is, in fact, no clearly safe dosage—all high-energy radiation, even of low intensity and brief duration, must be considered as potentially dangerous to the exposed individual.

Let us now turn to the effects of irradiation on the genes in the germ lines of exposed individuals. Here again we are handicapped by the special difficulties of dealing with the genetics of man, for the quantitative determination of the genetic effects of irradiation requires much more refined techniques than are possible with man—a point that becomes obvious when one tries to evaluate the data available concerning the survivors of the Hiroshima bomb. There is sufficient evidence that quantitative results obtained with one organism cannot safely be applied to a wholly different kind of organism. However, there are certain general qualitative results that have now been so widely confirmed that we may confidently assert that they apply to all higher organisms, including man. These results are:

- 1) High-energy irradiation produces mutations.
- 2) The frequency of induced mutations is directly proportional to the dosage of irradiation. There is almost certainly no threshold value below which irradiation is ineffective.
- 3) The effects of successive exposures are cumulative.
- 4) The effects are permanent in the descendants of the affected genes. There is no recovery.
- 5) The overwhelming majority of these mutations is deleterious—that is, they seriously affect the efficiency of individuals in later generations in which they come to expression. These deleterious genetic effects may lead to early death or to any of a wide variety of defects, often gross ones.

There is a store of such undesirable genes already present in any popula-

tion. What irradiation does is to add to this store.

It follows from these facts that any large-scale increase in the amount of irradiation to which human populations are subjected is a serious matter. Even though we cannot say that a given amount of irradiation will have a quantitatively specified effect, we can say that it will have some effect. The probability of an effect on the germ cells of any one individual may be very low; but when many millions of people are being exposed, it becomes certain that some of them will be affected. There is no possible escape from the conclusion that the bombs already exploded will ultimately result in the production of numerous defective individuals—if the human species itself survives for many generations. And every new bomb exploded, since its radioactive products are widely dispersed over the earth, will result in an increase in this ultimate harvest of defective individuals. Some such defectives would be present if the bombs had never been invented; the point is that the number due to the bombs will be added to this irreducible minimum.

Under these circumstances, I have been disturbed that Chairman Strauss of the Atomic Energy Commission should state, in an official press release from the White House, on 31 March 1954

. . . it should be noted that after every test we have had, and the Russian tests as well, there is a small increase in natural "background" radiation in some localities within the continental United States. But currently it is less than that observed after some of the previous continental and overseas tests, and far below the levels which could be harmful in any way to human beings. . . . [Bull. *Atomic Scientists* 10, 164 (May 1954)].

Presumably this statement is intended to refer only to immediate effects on exposed individuals; but, as I have pointed out, there are important other effects, less immediately apparent. Every geneticist familiar with the facts knows that any level whatever is certain to be at least genetically harmful to human beings when it is applied to most or all the inhabitants of the earth.

I do not wish to be understood as arguing that the benefits ultimately to be derived from atomic explosions are outweighed by the biological damage they do. It may be that the possible gains are worth the calculated risk. But it must be remembered that the risk is one to which the entire human race, present and future, is being subjected. I regret that an official in a position of such responsibility should have stated that there is no biological hazard from low doses of high-energy irradiation.



Reconstitution of Active Tobacco Mosaic Virus from Its Inactive Protein and Nucleic Acid Components*

H. FRAENKEL-CONRAT and ROBLEY C. WILLIAMS

Reprinted by authors' and publisher's permission from *Proceedings of the National Academy of Sciences*, vol. 41, 1955, pp. 690-698.

One of the most remarkable and exciting achievements in biological research in recent years was the reconstitution of active tobacco mosaic virus (abbreviated "TMV") from its inanimate constituent parts by Fraenkel-Conrat and Williams. While the distinction between "life" and "non-life" is a difficult thing to define at the level of the virus, this piece of research indicates that a substance considered to be living can be artificially constructed from substances considered to be non-living. Our bodies perform this transformation daily, of course, but it is the experimental reproduction of it that is impressive in this work.

Once more, in this paper, we see the importance of the introduction of new weapons into the arsenal of biology. The development of the electron microscope by the physicists found its greatest use and significance in its application to biological problems of ultra-structure, and it appears likely that it will do much to destroy the boundaries between physics and biology as distinct sciences, just as the study of the gene has done much to eliminate distinctions between chemistry and biology. At certain levels of biological investigation all boundaries disappear, and biology becomes a question of physico-chemical relationships.

MUCH RECENT EVIDENCE FROM CHEMICAL, PHYSICOCHEMICAL, ELECTRON MICROSCOPICAL, AND X-RAY STUDIES HAS RESULTED

IN A DEFINITE CONCEPT OF THE STRUCTURE OF THE TOBACCO MOSAIC VIRUS (TMV) PARTICLE.¹⁻⁵ IT APPEARS THAT ABOUT 2,800

* Aided by a grant from the National Foundation for Infantile Paralysis and research grant No. C-2245 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

¹ Harris, J. I., and Knight, C. A., *Nature* 170:613, 1952, and *J. Biol. Chem.* 214:231, 1955; Schramm, G., *Z. Naturforsch.*, 2b 112: 249, 1947; Watson, J. D., *Biochim. et biophys. acta* 13:10, 1954; Franklin, R., *Nature* 175: 379, 1955.

protein subunits of a molecular weight near 18,000 are arranged in a helical manner to form a rod with a hollow core. The nucleic acid is believed to occur as strands in the core. Electron micrographs which support this concept have been obtained of the virus at various stages of disaggregation.³⁻⁵ A protein isolated from infected plants has been found to reaggregate—first to short pieces of the presumed helix lying on end and resembling disks with central holes and then to much longer, but inactive, rods of the diameter of the virus yet free from nucleic acid.⁶ It has now been possible to achieve the co-aggregation of inactive virus protein subunits and inactive virus nucleic acid to give nucleoprotein rods which appear to be infective.

Preparation of Protein and Nucleic Acid Components.—TMV was dialyzed against pH 10–10.5 glycine buffer (0.01 M) or pH 10.5 carbonate-bicarbonate (0.1 M) at 3° C. for 48–72 hours. Undegraded virus was separated by cold ultracentrifugation, and the supernatant was brought to 0.4 saturation with ammonium sulfate. The protein alone precipitates (optical density_{260 mμ}/optical density_{280 mμ} = R = 0.65), leaving only nucleic acid (R = 2.0) in the supernatant; if this separation is not clean, longer alkali treatment is necessary. The protein moiety

is precipitated twice more with 0.25–0.35 saturated ammonium sulfate, dialyzed, brought to pH 7.0–8.0 with NaOH, and finally again freed from heavy particles, such as undegraded virus, by ultracentrifugation. The protein gives a water-clear solution at pH 7; the masked—SH group is still present. The spectrum resembles that of a mixture of tryptophan, tyrosine, cysteine, and phenylalanine, simulating the composition of the protein, although the minimum (at 250 mμ) is not quite as low (max./min. = 2.4 versus 2.9) (Fig. 1); P analyses (0.01–0.03 per cent) indicate removal of about 95–98 per cent of the nucleic acid. Evidence for the absence of detectable virus particles will be discussed below.

The nucleic acid fractions from such alkali-degraded TMV are not as effective for reconstitution as that obtained by the detergent method.² A virus solution (1 per cent) containing 1 per cent sodium dodecyl sulfate is adjusted to pH 8.5 and held at 40° for 16–20 hours. Following this treatment, ammonium sulfate is added to 0.35 saturation, and the protein precipitate is separated by centrifugation. When the supernate is refrigerated, from 60 to 90 per cent of the nucleic acid precipitates (R = 2.0) and is centrifuged off the next day, it is further purified by repeated resolution in ice water and precipitation with two volumes of cold ethanol and a few drops of 3 M pH 5 acetate. The nucleic acid solution is finally subjected to cold ultracentrifugation to remove any traces of virus. The virtual absence of protein is indicated by a minimum in O.D. near 230 mμ (max./min. = 3.0) (Fig. 1).

In a few preliminary ultracentrifuge experiments, kindly performed by Dr. Howard K. Schachman, the nucleic acid preparations exhibited one principal boundary with a sedimentation

² Fraenkel-Conrat, H., and Singer, B., *J. Am. Chem. Soc.* 76:180, 1954.

³ Schramm, G., Schumacher, G., and Zillig, W., *Nature* 175:549, 1955.

⁴ Rice, R. V., Kaesberg, P. and Stahmann, M. A., *Biochim. et biophys. acta* 11:337, 1953.

⁵ Hart, R. G., these *Proceedings* 41:261, 1955.

⁶ Takahashi, W. N., and Ishii, M., *Nature* 169:419, 1952; Delwiche, C. C., Newmark, P., Takahashi, W. N., and Ng, M. J., *Biochim. et biophys. acta* 16:127, 1955; Commoner, B., Yamada, M., Rodenberg, S. D., Wang, F. Y., and Basler, E. Jr., *Science* 118: 529, 1953.

coefficient of about 8 S, resembling the preparation of Cohen and Stanley.⁷ The protein, in pH 9 borate (0.01 M) or in 0.01 M NaCl at that pH, exhibited largely a single boundary with a sedimentation coefficient of 4.5 S. At lower pH values larger components, presumably due to aggregation, were observed.

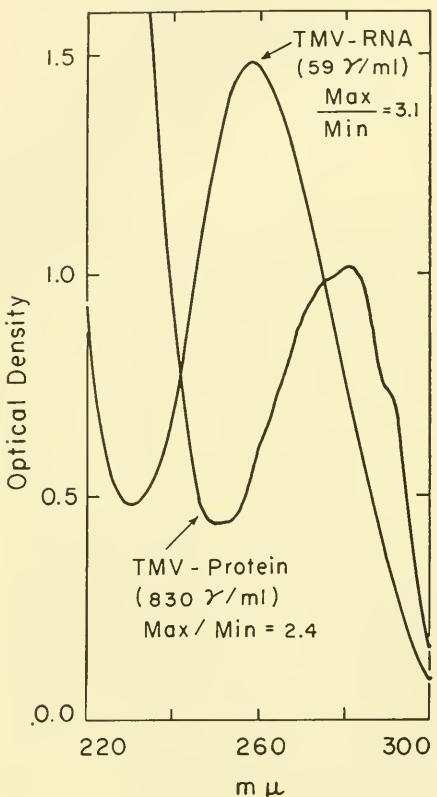


Fig. 1. Ultraviolet absorption spectrum of purified virus protein and nucleic acid.

Reconstitution of Active Virus.—For reconstitution of the virus, 1 ml. of approximately 1 per cent protein solution is mixed with 0.1 ml. of a 1 per cent nucleic acid solution. Opalescence appears after addition of a suit-

able buffer; 0.01 ml. of pH 6 acetate (3 M) has given the best results, but phosphate (pH 6.3 and 7.0) and pH 6 ammonium acetate have also been used successfully. The samples are held at 3° for at least 24 hours. They may then be directly diluted and assayed. More often they were ultracentrifuged, the pellets redissolved in water, traces of insoluble material separated by centrifugation, and aliquots of the opalescent supernatants diluted for spectrophotometry. Most of the protein and 40–60 per cent of the nucleic acid⁸ were in the pellet, and the spectrum was that expected for a nucleoprotein such as TMV (max./min. = about 1.17). On the basis of the O.D. of TMV (0.27 at 260 mμ for an 0.01 per cent solution), approximate concentrations were calculated, and the sample was diluted to a range of 100 to 10 µg./ml. for assay. For pellets composed of protein only (absorption maximum at 280 mμ), calculations were based on the O.D. of the virus proteins (0.13 for an 0.01 per cent solution at that wave length). Assays were performed on groups of 8–12 plants (*Nicotiana glutinosa*), distributing each of an equal number of samples (including at least one standard and often a solvent blank) over 10 equivalent half-leaves. It was indeed quite surprising to find that the reconstituted nucleoprotein preparations produced local lesions (from 2 to 30 per half-leaf), when tested at 10–100 µg./ml., which were indistinguishable in appearance from those of TMV at 0.1 µg./ml. In contrast, no activity was observed when the protein or nucleic acid alone was tested up to 1,800

⁷ Cohen, S. S., and Stanley, W. M., *J. Biol. Chem.* 142:863, 1942.

⁸ When less than 6 per cent of nucleic acid is added to the protein, almost all is incorporated in the pellet, but lower infectivity is the result. Protein alone also forms pellets upon ultracentrifugation at pH 6.

and 52 µg./ml., respectively.⁹ When 0.005 per cent TMV was held for several hours in 5 per cent protein or 0.5 per cent nucleic acid, it was found fully active upon dilution and assay. This strongly suggests that the noninfectivity of the two components cannot be attributed to inhibition phenomena (Table 1).

TABLE 1

ACTIVITY OF THREE PREPARATIONS OF RECONSTITUTED VIRUS AND OF TMV AT DIFFERENT ASSAY LEVELS; ABSENCE OF INHIBITORS FROM COMPONENTS

MATERIAL TESTED	ASSAYS µg./Ml.	Lesions/Half-Leaf*		
		27	54	31
Reconstituted virus: 25,000-rpm. pellet	100	—	—	120†
	30	18	—	—
	25	—	35	—
	10	9	7	14†
40,000-rpm. pellet	100	2	23	—
TMV stock preparation	0.25	27	—	—
	15	—	—	—
	0.1	9 (6.1-12.1)‡	—	—
	0.02	5	—	—
TMV-protein added at 1000:1	0.1	10	—	—
TMV-RNA added at 100:1	0.1	5	—	—
		13	—	—

* Each figure is the average of 10 equivalent half-leaves. The 3 columns in top half of table refer to separate preparations.

† This is one of the preparations which appeared to increase in activity during storage.

‡ Average and range of 9 assays (10 half-leaves each) of one TMV preparation over 3 months.

at room temperature appears to be required for the formation of any active rods. These experiments represent con-

⁹ The usual assay method yielded usually 0.0, but at times up to 0.4, lesions per half-leaf when phosphate alone was applied. This result is probably due to accidental contamination by active material applied to other sites of the plant. Therefore, separate plants were used when the absence of active virus was in question. Standard TMV was applied to one bottom leaf and the unknowns to all others. This gave consistently 0.0 lesions with the components used in virus reconstitution, except when the nucleic acid was applied at 200 µg./ml., twenty times the amount present at the highest assay level of the reconstituted virus (0.3 lesions per half-leaf).

vincing control data; they also seem to prove that we are dealing with a definite chemical reaction and to exclude most other interpretations. The observation that the nucleic acid gradually loses most of its activity during several weeks of storage at 3° (in aqueous solution, pH 5) also serves as a control experiment, indicating the crucial nature of its physical state. Treatment of the nucleic acid (600 µg.) with ribonuclease (0.2 µg.) in 10⁻⁴ M magnesium sulfate rendered it unable to combine with the protein to produce active rods; also, substitution of other nucleic acids (DNA from

thymus and RNA from turnip yellow mosaic virus¹⁰) yielded almost nucleic acid-free pellets which were inactive (Table 2).

A few preliminary experiments on the properties of the reconstituted virus indicated somewhat greater lability to alkali (pH 9) than that of TMV.

TABLE 2

EFFECT OF REACTION CONDITIONS ON REGENERATION OF VIRUS ACTIVITY

REACTION CONDITIONS *	ASSAYS	
	μg./Ml.	Lesions/Half-Leaf
Protein (1 per cent) + RNA (0.1 per cent):		
1 minute, pH 6	100	0.6
24 hours	100	10.2, 13.1†
96 hours	100	13.0
Protein (0.01 per cent) + RNA (0.001 per cent):		
24 hours, pH 6	100	0.6, 0.2†
96 hours	100	0.1, 0.2†
Protein + RNase-digested RNA	100	0.0
Protein + TYMV-RNA	100	0.3
Protein ‡	500; 625; 1800	0.0; 0.0; 0.0
RNA ‡	30; 52; 230	0.0; 0.0; 0.3

* Protein and RNA stand for the preparations isolated from TMV by the methods described in the text. TYMV-RNA is the ribonucleic acid isolated from turnip yellow mosaic virus. RNase is ribonuclease.

† Reassayed dilute solution after one week.

‡ Different preparations, assayed about 10 times, generally on separate plants (see n. 9); in the customary half-leaf assay method, up to 0.5 lesions were occasionally obtained with the same preparations.

Ribonuclease (0.2 μg. for 5 mg.) also appeared to decrease the activity. A fractionation of the nucleoprotein material by centrifugation at 25,000 rpm. for 30 minutes yielded a pellet of considerably higher infectivity than the residual half of the material, pelleted at 40,000 rpm. (1 hour). Material so purified seemed to increase in activity upon storage in 0.3–0.5 per cent solution at 3°. It also appeared to become more resistant to alkali and to ribonuclease.

Electron Microscopic Studies.—Electron microscopy was used qualitatively to study the shapes and sizes of the components involved in the formation of the virus rods and quantitatively¹¹

to assess the degree of homogeneity of the suspensions as well as to obtain counts and length distributions of the reconstituted rods. In the protein, the only visible particles, aggregates presumably of the original 4.5 S material, appeared as disks about 5–15 mμ thick and with central holes. The diameter of the disk was 15 mμ and that of the central hole about 4 mμ (Fig. 2).

The appearance of these perforated disks is identical with that of partially polymerized X-protein.⁵ In the nucleic acid solution only occasional poorly defined fibrils could be detected. The reconstituted rods appeared to be identical in shape and size with intact TMV, except for a greater randomness of length (Fig. 3).

Preparations of the purified protein and nucleic acid were examined for any electron microscopic evidence of contamination with intact TMV. In a

¹⁰ Kindly supplied by Dr. S. S. Cohen and Dr. V. Cosentino.

¹¹ Williams, R. C., Backus, R. C., and Steere, R. L., *J. Am. Chem. Soc.* 73:2062, 1951.

specific instance the protein was sprayed upon the specimen screens at a concentration of 0.1 per cent and the RNA at 0.007 per cent. These are tenfold and sevenfold greater concentrations of the two components than the highest levels used in the assays of the reconstituted virus (100 $\mu\text{g./ml.}$). The protein preparation showed no rods of a length even as great as 100 $\text{m}\mu$ in six droplet patterns each of which had a volume of approximately $3 \times 10^{-9} \text{ ml.}$ The RNA material also showed no rods in six droplets of approximately this average volume. From these fig-



Fig. 2. Electron micrograph of the TMV protein used in the reconstitution experiments. The particles are characteristically disk-shaped, with central holes. X120,000.

ures it can be calculated that the constituent protein and RNA solutions contribute fewer than 5×10^7 typical

TMV particles per milliliter to the reconstituted virus in the highest concentration assayed. Additional counts



Fig. 3. Particles of the reconstituted tobacco mosaic virus. Their morphology is identical with that of normal TMV, except for a greater proportion of short particles. The longest rod in this field is about 300 $\text{m}\mu$ long. X60,000.

made on the standard TMV used as infectivity control showed that approximately 7×10^8 particles per milliliter, of typical length, are required to produce the 10 lesions per leaf customarily obtained. Inasmuch as several preparations of the reconstituted virus gave about twice that number of lesions at the 100 $\mu\text{g./ml.}$ level, we can conclude that the starting materials failed by at least a factor of 30 to contain enough contaminating TMV particles to account for the final infectivity. This conclusion rests upon the

supposition that the specific infectivity of any TMV particles that might remain as contaminants in the protein and RNA preparations is the same as that possessed by the untreated control TMV.

Counts and length distributions were secured for the particles of both TMV

and the most active reconstituted virus, the 25,000-rpm. pellet described above, in order to ascertain the relative biological efficiency of the two materials in terms of numbers of particles per milliliter per lesions per leaf. The results of the counts and length distributions are given in Table 3. From this

TABLE 3

ELECTRON MICROSCOPE COUNTS OF PARTICLES OF TMV AND RECONSTITUTED VIRUS
AT ASSAY CONCENTRATION

Preparation	Assay Concentration ($\mu\text{g./Ml.}$)	Lesions/ Half-Leaf	Total Particles/Ml.	Particles/Ml. of Length 290-310 M μ	Particles/Ml./ 10 Lesions
Control TMV	0.1	10	1.6×10^9	7.0×10^8	7×10^8
Reconstituted virus (25,000 rpm.)	10	10	2.2×10^{11}	2.0×10^{10}	2×10^{10}

table it appears that about one-tenth of the total particles in the reconstituted virus, representing about one-third of the total mass, were of length *ca.* 300 m μ , the length of the monomer of normal TMV. These particles were therefore only about 3 per cent as infective as the particles of similar length in the control TMV. It thus appears that polymerization of the protein by nucleic acid to form ~300-m μ rods is fairly frequent but that only a fraction of the rods is reconstituted with the structural faithfulness necessary for infectivity.

The length distribution of the rods of this pelleted material exhibited a reasonably random character for lengths less than about 260 m μ , but a highly uniform length between 290 and 310 m μ for the 10 per cent of the rods falling in this range. Only 3 per cent of the particles were of lengths greater than 310 m μ . This is in contrast to aggregates of X-protein, which show a complete randomness of distribution of lengths.

To ascertain whether the nucleic acid was localized in the center of the newly formed virus rods, Dr. R. Hart applied his technique of detergent



Fig. 4. Particles of reconstituted TMV treated briefly with hot detergent. Rods are seen with strands of nucleic acid projecting from their ends. This appearance is identical with that found for normal TMV more severely treated. X55,000.

treatment, followed by electron microscopic analysis.⁵ He found the reconstituted rods appreciably more labile to SDS than was standard TMV, but after SDS treatment for 10 seconds many rods were partially degraded and showed a central strand of material protruding from the ends, as does standard TMV after 60 seconds of reaction (Fig. 4). These strands disappeared when ribonuclease was added.

Summary.—The preparation from TMV of protein and RNA fractions which tend to recombine at about pH 6 to form a nucleoprotein carrying virus activity (0.1–1 per cent of that of TMV) is described.

An electron microscopic search revealed no TMV rods in either of the two component solutions at a concentration level thirty fold to three hundred fold greater than those at which the reconstituted virus was assayed. In the latter, on the other hand, up to about one-third of the material con-

sisted of rods of the typical diameter and length of TMV, many, if not all, containing a nucleic acid core.

The concentration, time, and pH dependence of virus regeneration is that of a typical chemical reaction. Freshly prepared RNA is required for appreciable reaction; degraded RNA, or nucleic acids from other sources, are inactive.

No inhibition was observed if known small amounts of TMV were added to concentrated solutions of each of the components and subsequently diluted and assayed.

The evidence thus seems reasonably complete that, under the conditions described, TMV nucleic acid enters into combination with TMV protein subunits and favors aggregation to rods, some of which are of sufficient length and structural integration to carry infectivity.

The capable assistance of Mrs. B. Singer and Mr. J. Toby is gratefully acknowledged.



Fine Structure of a Genetic Region in Bacteriophage*

SEYMOUR BENZER

Reprinted by author's and publisher's permission from *Proceedings of the National Academy of Sciences*, vol. 41, 1955, pp. 344–354.

The technique introduced in this paper, which provides a method of measuring the actual size of a genetic unit by the way the organism reacts in specified experimental situations, appears to be quite

* Supported by a grant-in-aid from the American Cancer Society upon recommenda-

tion of the Committee on Growth of the National Research Council.

successful as a way to determine the detailed structure of the gene. When this information is combined with the model of the DNA molecule provided by Watson and Crick (p. 241), and the knowledge provided by Fraenkel-Conrat and Williams (p. 264) on the reunion of particles to form a functional virus, it can be seen that the science of genetics is on the verge of a major break-through, if it hasn't already completed it. Slightly more than 50 years of work has brought the science to this point. It will be fascinating to review the classics of the field in a similar fashion 50 years hence to see where it has gone, and who has led it there.

THIS PAPER DESCRIBES A FUNCTIONALLY related region in the genetic material of a bacteriophage that is finely subdivided by mutation and genetic recombination. The group of mutants resembles similar cases which have been observed in many organisms, usually designated as "pseudo-alleles." (See reviews by Lewis¹ and Pontecorvo.²) Such cases are of special interest for their bearing on the structure and function of genetic determinants.

The phenomenon of genetic recombination provides a powerful tool for separating mutations and discerning their positions along a chromosome. When it comes to very closely neighboring mutations, a difficulty arises, since the closer two mutations lie to one another, the smaller is the probability that recombination between them will occur. Therefore, failure to observe recombinant types among a finite number of progeny ordinarily does not justify the conclusion that the two mutations are inseparable but can only place an upper limit on the linkage distance between them. A high degree of resolution requires the examination of very many progeny. This can best be achieved if there is available a selective feature for the detection of small proportions of recombinants.

¹ Lewis, E. B., *Cold Spring Harbor Symposia Quant. Biol.* 16:159-174, 1951.

² Pontecorvo, G., *Advances in Enzymol.* 13:121-149, 1952.

Such a feature is offered by the case of the rII mutants of T4 bacteriophage described in this paper. The wild-type phage produces plaques on either of two bacterial hosts, B or K, while a mutant of the rII group produces plaques only on B. Therefore, if a cross is made between two different rII mutants, any wild-type recombinants which arise, even in proportions as low as 10^{-8} , can be detected by plating on K.

This great sensitivity prompts the question of how closely the attainable resolution approaches the molecular limits of the genetic material. From the experiments of Hershey and Chase,³ it appears practically certain that the genetic information of phage is carried in its DNA. The amount of DNA in a particle of phage T2 has been determined by Hershey, Dixon, and Chase⁴ to be 4×10^5 nucleotides. The amount for T4 is similar.⁵ If we accept the model of DNA structure proposed by Watson and Crick,⁶ consisting of two paired nucleotide chains, this corresponds to a total length of DNA per T4 particle of 2×10^5 nu-

³ Hershey, A. D., and Chase, M., *J. Gen. Physiol.* 36:39-56, 1952.

⁴ Hershey, A. D., Dixon, J., and Chase, M., *J. Gen. Physiol.* 36:777-789, 1953.

⁵ Volkin, E. K., personal communication.

⁶ Watson, J. D., and Crick, F. H. C., *Cold Spring Harbor Symposia Quant. Biol.* 18: 123-131, 1953.

cleotide pairs. We wish to translate linkage distances, as derived from genetic recombination experiments, into molecular units. This cannot be done very precisely at present. It is not known whether all the DNA in a phage particle is indispensable genetic material. Nor is it known whether a phage "chromosome" (i.e., the physical counterpart of a linkage group identified by genetic means) is composed of a single (duplex) DNA fiber or whether genetic recombination is equally probable in all chromosomal regions. For the purpose of a rough calculation, however, these notions will be assumed to be true. Thus we place the total linkage map of T4 in correspondence with 2×10^5 nucleotide pairs of DNA. The total known length of the three linkage groups⁷ in phage T4 amounts to some 100 units (one unit = 1 per cent recombination in a standard cross). In addition, there is evidence⁸ for roughly another 100 units of length connecting two of the groups. Therefore, if we assume 200 recombination units to correspond to 2×10^5 nucleotide pairs, the recombination per nucleotide pair is 10^{-3} per cent. That is to say, given two phage mutants whose mutations are localized in their chromosomes at sites only one nucleotide pair apart, a cross between these mutants should give rise to a progeny population in which one particle in 10^5 results from recombination between the mutations (provided, of course, that recombination is possible between adjacent nucleotide pairs). This computation is an exceedingly rough one and is only intended to indicate the order of magnitude of the scale factor. Some preliminary results are here presented of a program de-

signed to extend genetic studies to the molecular (nucleotide) level.

r Mutants.—The wild-type phages T2, T4, and T6 produce small plaques with rough edges when plated on strain B of *Escherichia coli*. From sectors of clearing in these plaques, mutants can be readily isolated which produce large, sharp-edged plaques (Hershey⁹). These mutants have been designated "r" for rapid lysis; they differ from the wild type by a failure to cause "lysis inhibition" on strain B (Doermann¹⁰). The wild type has a selective advantage over r mutants when the two types grow together on B. The genetics of r mutants was studied by Hershey and Rotman,¹¹ who found three regions in the linkage map of T2 in which various mutations causing the r phenotype were located, including one large "cluster" of mutants which were shown to be genetically distinct from one another. The genetic study of T4 by Doermann and Hill⁷ showed r regions corresponding to two of those in T2. T6 also has at least two such r regions.

The rII Group.—For all three phases, T2, T4, and T6, the r mutants can be separated into groups on the basis of their behavior on strains other than B. This paper will be concerned only with one group, which will be called the "rII group." Mutants of the rII group are distinguished from those of other groups, and from wild type, by a failure to produce plaques on certain lysogenic strains¹² of *E. coli* which carry phage λ . As shown in Table 1, a mutant of the rII group

⁹ Hershey, A. D., *Genetics* 31:620-640, 1946.

¹⁰ Doermann, A. H., *J. Bacteriol.* 55:257-276, 1948.

¹¹ Hershey, A. D., and Rotman, R., *Genetics* 34:44-71, 1949.

¹² Lederberg, E. M., and Lederberg, J., *Genetics* 38:51-64, 1953.

⁷ Doermann, A. H., and Hill, M. B., *Genetics* 38:79-90, 1953.

⁸ Streisinger, G., and Bruce, V., personal communication.

TABLE 1

PHENOTYPE PLAQUE MORPHOLOGY OF T4
WILD AND rII MUTANT PLATED ON
VARIOUS HOSTS

	HOST STRAIN		
	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
T4 wild type	B	K12S	K12S (λ)
T4 rII mutant	Wild	Wild	Wild

produces r-type plaques on strain B, wild-type plaques on strain K12S (nonlysogenic strain sensitive to λ), and no plaques on K12S (λ) (derived from K12S by lysogenization with λ). The wild-type phage produces similar plaques on all three strains. In the case of T4, with which we shall be concerned in this paper, the efficiencies of

plating are approximately equal on the three strains, except, of course, for rII on K12S (λ). The three bacterial strains will be here designated as "B," "S," and "K."

Approximately two-thirds of the independently arising r mutants isolated on B are of the rII type. This group includes the "cluster" of r mutants of T2 described by Hershey and Rotman and the r47 and r51 mutants described by Doermann and Hill in the corresponding map region of T4 but does not include r mutants located outside that region. Similarly, all newly isolated mutants showing the rII character have turned out to fall within the same region, as indicated in Figure 1.

The properties of the rII group are

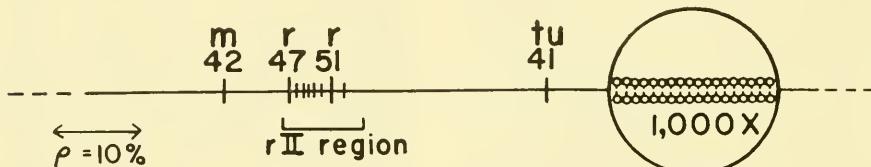


Fig. 1. Partial linkage map of T4 (Doermann), indicating the location of the rII region. *m* and *tu* designate "minute plaque" and "turbid plaque" mutations. The circular inset shows, diagrammatically, the corresponding dimensions of the DNA chain magnified 1,000 diameters.

especially favorable for detailed genetic study. An rII mutant has three different phenotypes on the three host strains (Table 1): (1) altered plaque morphology on B, (2) indistinguishable from wild type on S, and (3) unable to produce plaques on K. These properties are all useful. By virtue of their altered plaque type on B, r mutants are readily isolated, and those of the rII group are identified by testing on K. Where it is desired to avoid a selective disadvantage compared with wild type, e.g., in measuring mutation rates, S can be used as a nondiscriminating host. The failure of rII mutants to plate on K enables one to detect

very small proportions of wild-type particles due to reversion or due to recombination between different rII mutants.

Fate of rII Mutants in K.—Wild-type and rII mutants adsorb equally well to strains S and K. Whereas the wild type provokes lysis and liberation of a burst of progeny on both strains, the rII mutant grows normally only on S. Infection of K with an rII mutant provokes very little (and/or very late) lysis, although all infected cells are killed. The block in growth of rII mutant is associated with the presence of the carried phage λ . The reason for this association is unknown.

Quantitative Differences in Phenotype.—While all rII mutants show the same phenotypic effect of poor multiplication on K, they differ in the degree of this effect. A certain proportion of K infected with rII actually liberates some progeny, which can be detected by plating the infected cells on B. The fraction of infected cells yielding progeny defines a "transmission coefficient" characteristic of the mutant. The transmission coefficient is insensitive to the multiplicity of infection but depends strongly upon the physiological state of the bacteria (K) and upon temperature. Under given conditions, however, the coefficient can be used as a comparative index of degree of phenotypic effect, a "leaky" mutant having a high coefficient. As can be seen in Table 2, a wide range of values is found.

TABLE 2

PROPERTIES OF T4 MUTANTS OF THE
rII GROUP *

Mutant Number	Map Position	Transmission Coefficient	Reversion Index (units of 10^{-6})
r47	0	.03	<0.01
r104	1.3	.91	<1
r101	2.3	.03	4.5
r103	2.9	.02	<0.2
r105	3.4	.02	1.8
r106	4.9	.55	<1
r51	6.7	.02	170
r102	8.3	.02	<0.01

* Three parameters are given for each mutant. The map position is computed from the sum of the nearest intervals shown in Figure 2 and is given in percentage recombination units, taking the position of r47 as zero. The "transmission coefficient" is a measure of phenotypic effect determined by infecting bacteria K with the mutant in question and is given as the fraction of such infected cells yielding plaques on strain B. The "reversion index" is the average fraction of wild-type particles arising in lysates of the mutant grown from a small inoculum on a non-selective host.

Plaques on K.—Some rII mutants produce no plaques on K, even when as many as 10^8 particles (as measured by plaque count on B) of a stock are plated. Other rII mutants, however, produce various proportions of plaques on K. When the plaques appearing on K are picked and retested, they fall into three categories: (1) a type which, like the original mutant, produces very few plaques on K and r-type plaques on B; (2) a type which produces plaques (often smaller than wild type) on K with good efficiency but r-type plaques on B; and (3) a type indistinguishable from the original wild. These three types are understood to be due to the following: (1) "leaking" effects, i.e., ability of the mutant to grow slightly on K, so that there is a chance for a few visible plaques to form; (2) a mutation which partially undoes the effect of the rII mutation, so that multiplication in K is possible, but the full wild phenotype is not achieved; and (3) apparent reverse mutation, which may or may not be genuine, to the original wild type.

The proportion of each type occurring in a stock is characteristic and reproducible for a particular rII mutant but differs enormously from one rII mutant to another. There is no evident correlation in the rates of occurrence of the three types.

Reversion Rates of rII Mutants.—Reversion of r mutants to a form indistinguishable from wild type was demonstrated by Hershey,⁹ who made use of the selective advantage of wild type on B to enrich its proportion in serial transfers. Given the inability of rII mutants to produce plaques on K, such reversions are easily detected, even in very small proportion. An index to the frequency of reversion of a particular rII mutant can be obtained by preparing a lysate from a small inoculum (about 100 particles, say, so that there

is very little chance of introducing a wild-type particle present in the stock). If S is used as the host, both rII mutant and any reversions which arise can multiply with little selection, as shown by control mixtures. The average fraction of wild-type particles present in several lysates is an index which can be shown to be roughly proportional to the probability of reversion per duplication of the rII mutant. Under the conditions of measurement the index is of the order of 10–20 times the probability of reversion per duplication. The plaques appearing on K must be tested by picking and replating on B. This eliminates the "spurious" plaques produced by partial reversions and by leaky mutants, which show up as r type on B. As may be seen in Table 2, the reversion indices for rII mutants vary over a very wide range. One mutant has been found which reverts 10 times more frequently than r51, so that the reversion rates cover a known range of over 10⁵-fold.

It has not been proved that these apparent reversions constitute a genuine return to the original wild type. However, the possibility of suppressor mutations distant from the site of the rII mutation has been ruled out by backcrosses to the original wild type. Krieg¹³ found very few, if any, r-type recombinants in backcrosses of several reversions, localizing the reverse changes to within a few tenths of a per cent linkage distance from the original rII mutations. One case of "partial reversion" has also been tested by backcrossing, and failure to observe rII-type recombinants localized the "partial reverse mutation" to within the rII region.

Mapping of the rII Region.—A cross between two rII mutants is made by

infecting a culture of B with equal multiplicities (three per bacterium) of each type. The yield after lysis contains the two parental types and, if the parents are genetically distinct, two recombinant types, the double mutant and wild type. In the average yield from many cells, the recombinant types occur in equal numbers.¹¹ In all cases thus far tested, double rII mutants, like single mutants, do not produce plaques on K. On the assumption that this is generally true, the proportion of recombinants in the yield can be measured simply by doubling the ratio of the plaque count on K (which registers only the wild recombinant) to the count on B (which registers all types). The percentage of wild type thus measured agrees well with a direct count of plaque types on B.

In this way, a series of six rII mutants of T4 (the first six isolated—not selected in any way) have been crossed with each other and with r47 and r51 (kindly supplied by A. H. Doermann) in 23 of the 28 possible pairs. The results of these crosses are given in Figure 2 and are compatible with the indicated seriation of the mutants. The dis-

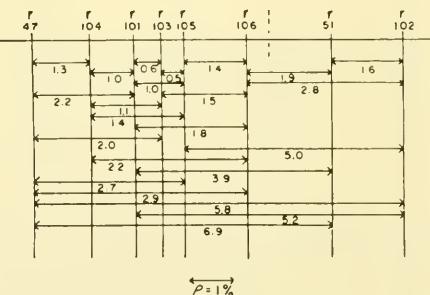


Fig. 2. Larger-scale map of eight rII mutants, including Doermann's r47 and r51. Newly isolated mutants are numbered starting with 101. The recombination value (in per cent) for each cross is obtained by plating the progeny on K and on B and doubling the ratio of plaque count on K to count on B.

¹³ Krieg, D., personal communication.

tances are only roughly additive; there is some systematic deviation in the sense that a long distance tends to be smaller than the sum of its component shorter ones. Part of this discrepancy is accounted for by the Visconti-Delbrück correction for multiple rounds of mating.¹⁴ Reversion rates were small enough to be negligible in these crosses. Thus, while all rII mutants in this set fall into a small portion of the phage linkage map, it is possible to seriate them unambiguously, and their positions *within* the region are well scattered.

Tests for Pseudo-allelism.—The functional relatedness of two closely linked mutations causing similar defects may be tested by constructing diploid heterozygotes containing the two mutations in different configurations.^{1,2} The *cis* form, with both mutations in one chromosome, usually behaves as wild type, since the second chromosome supplies an intact functional unit (or units). However, the *trans* form, containing one of the mutations in each chromosome, may or may not produce the wild phenotype. If it does, it is concluded that the two mutations in question are located in separate functional units.

In applying this test to the rII mutants, the diploid heterozygote can be simulated by a mixed infection with two kinds of phage. The rII phenotype is a failure to lyse K, whereas the wild phenotype is to cause lysis. If K is mixedly infected with wild type and rII mutant, the cells lyse, liberating both types of phage. Thus the presence of wild type in the cell supplies the function which is defective in rII type, and the rII mutation can be considered "recessive." Although it has not yet been tested, the *cis* configura-

tion of double rII mutant plus wild type is also presumed to produce lysis in all cases. The *trans* configuration is obtained by infecting K with the pair of rII mutants in question. This is found to give lysis or not, depending upon which rII mutants compose the pair. The results are summarized by the dotted line in Figure 2, indicating a division of the rII region into two segments. If both mutants belong to the same segment, mixed infection of K gives the mutant phenotype (very few cells lyse). If the two mutants belong to different segments, extensive lysis occurs with liberation of both infecting types (and recombinants). These results are summarized in Figure 3. Thus, on the basis of this test, the two segments of the rII region correspond to independent functional units.

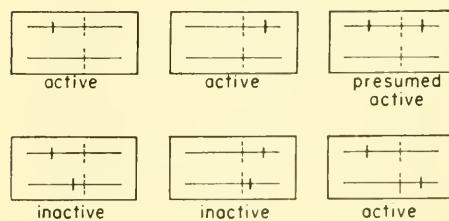


Fig. 3. Summary of tests for "position-effect pseudo-allelism" of rII mutants. Each diagram represents a diploid heterozygote as simulated by mixed infection of a bacterium (K) with two types of phage containing the indicated mutations. *Active* means extensive lysis of the mixedly infected cells; *inactive* means very little lysis. The dotted line represents a dividing point in the rII region, the position of which is defined by these results.

Actually, for mixed infection of K with two (nonleaky) mutants of the *same* segment, a very small proportion of the cells do lyse and liberate wild recombinants, that proportion increasing with the linkage distance between

¹⁴ Visconti, N., and Delbrück, M., *Genetics* 38:5-33, 1953.

the mutations. For two rII mutants separated by 1 per cent linkage distance (measured by a standard cross on B) the proportion of mixedly infected K yielding any wild particles is about 0.2 per cent.

This value has bearing upon the effect upon K/B values of the heterozygous phage particles which arise in a cross between two rII mutants on B. In such a cross between closely linked rII mutants, the progeny should include about 2 per cent of particles containing a *trans* configuration heterozygous piece.¹⁵ When one of these is plated on K, there is a certain chance that a wild recombinant may form in the first cycle of infection, leading to production of a plaque. If it is assumed that these are no more likely to do so than a mixed infection of K with two complete mutant particles, it can be concluded that the effect of these heterozygous particles upon the count on K is negligible, provided that both rII mutants belong to the same segment. For mutants in different segments, however, the "efficiency" of the heterozygous particles should be much greater, and recombination values measured by the K/B method should run considerably higher than the true values. The recombination values in Figure 2 for crosses which transgress the segmental divide are probably subject to some correction for this reason.

Rough Mapping by Spot Test.—If a stock of either of two rII mutants is plated on K, no plaques arise; but if both are plated together, some bacteria become infected by both mutants and, if this leads to the occurrence of wild-type recombinants,

plaques are produced. If the two mutants are such that wild recombinants cannot arise between them (e.g., if they contain identical mutations), no plaques appear. A given rII mutant may thus be tested against several others on a single plate by first seeding the plate with K plus the mutant in question (in the usual soft agar top layer) and then spotting with drops containing the other rII mutants.

Inspection of such a plate immediately places the unknown mutant in the proper segment, since spotting any mutant of segment A against any mutant of segment B gives a very clear spot, due to the extensive lysis of mixedly infected bacteria. However, for a pair of mutations belonging to the same segment, plaques are produced only by the relatively few mixedly infected bacteria which give rise to wild recombinants. The greater the linkage distance between the mutations, the larger the number of plaques that appear in the spot. A group of mutants of the same segment may thus be seriated by seeding one plate with each and spotting with all the others. Given a previously seriated group, a new mutant can thus be quickly located within the group. This method works best for mutants which are stable (i.e., low reversion rate) and nonleaky, so that large numbers of phage particles can be plated. Reversions or pronounced leaking effects obviously cause an obscuring background.

This test has been applied to a large group of stable, nonleaky rII mutants. Their approximate locations as deduced from these tests are shown in Figure 4. Some of the mutants showed anomalies which made it impossible to locate them as members of a series. They gave very little recombination with any of the mutants located within a certain span, while behaving nor-

¹⁵ Hershey, A. D., and Chase, M., *Cold Spring Harbor Symposia Quant. Biol.* 16: 471-479, 1951; Levinthal, C., *Genetics* 39:169-184, 1954.

mally with respect to mutants located outside that span. They are indicated

in Figure 4 by horizontal lines extending over the span.

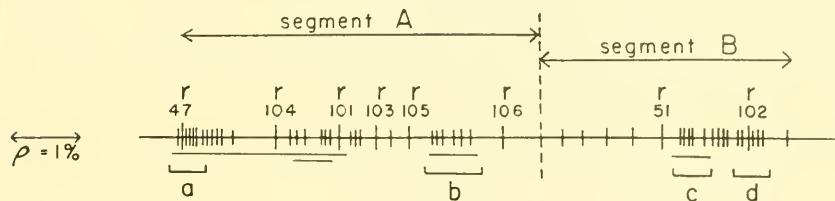


Fig. 4. Preliminary locations of various *r* II mutants, based upon spot tests.

Spot tests on numerous other mutants have shown that mutants of varied reversion rates, transmission coefficients, and rates of "partial reversion" occur at scattered positions in both segments.

Mapping of "Microclusters."—The spot test enables us to pick out "microclusters," i.e., groups of very closely neighboring mutations. Four such groups selected for further study are indicated in Figure 4, and the results of mapping them are given in Figure 5. While some intervals show reasonably good additivity properties, there are some mutants which give violently anomalous results. Thus in microcluster *a*, *r*47 gives no wild recombinants (i.e., less than 1 in 10^6) with any of the other three mutants, but two pairs of the three do show recombination. These results can be understood if it is assumed that each mutation extends over a certain length of the chromosome, and production of wild type requires recombination within the space between those lengths. According to this interpretation, the mutations would cover the lengths indicated by the bars in Figure 5. These anomalies resemble those observed in the spot tests, only they are more limited in span.

This observation raises the question of whether there exist true "point" mutations (i.e., involving an alteration of only one nucleotide pair) or

whether all mutations involve more or less long pieces of the chromosome. It must be remembered that the mutants used in these experiments were selected for extreme stability against reversion. This procedure would be expected to enrich the proportion of mutants containing gross chromosomal alterations. So far as is known, the anomalous cases observed could equally well be imagined to be due to double (i.e., two near-by "point") mutations, inversions, or deletions of the wild-type chromosome. In continuing these experiments, it would seem well advised to employ only mutants for which some reversion is observed.

Discussion.—The set of *r*II mutants defines a bounded region of a linkage group in which mutations may occur at various locations, all the mutations leading to qualitatively similar phenotypic effects. The *r*II region would seem, therefore, to be functionally connected, so that mutations arising anywhere within the region affect the same phenotype. This effect is expressed, in case strain B is the host, by failure to produce lysis inhibition; in case S is the host, by no consequence; and in case K is the host, by inability to multiply normally. The failure of an *r*II mutant to mature in K can be overcome by the presence of a wild-type phage in the same cell. This could be understood if the function of the

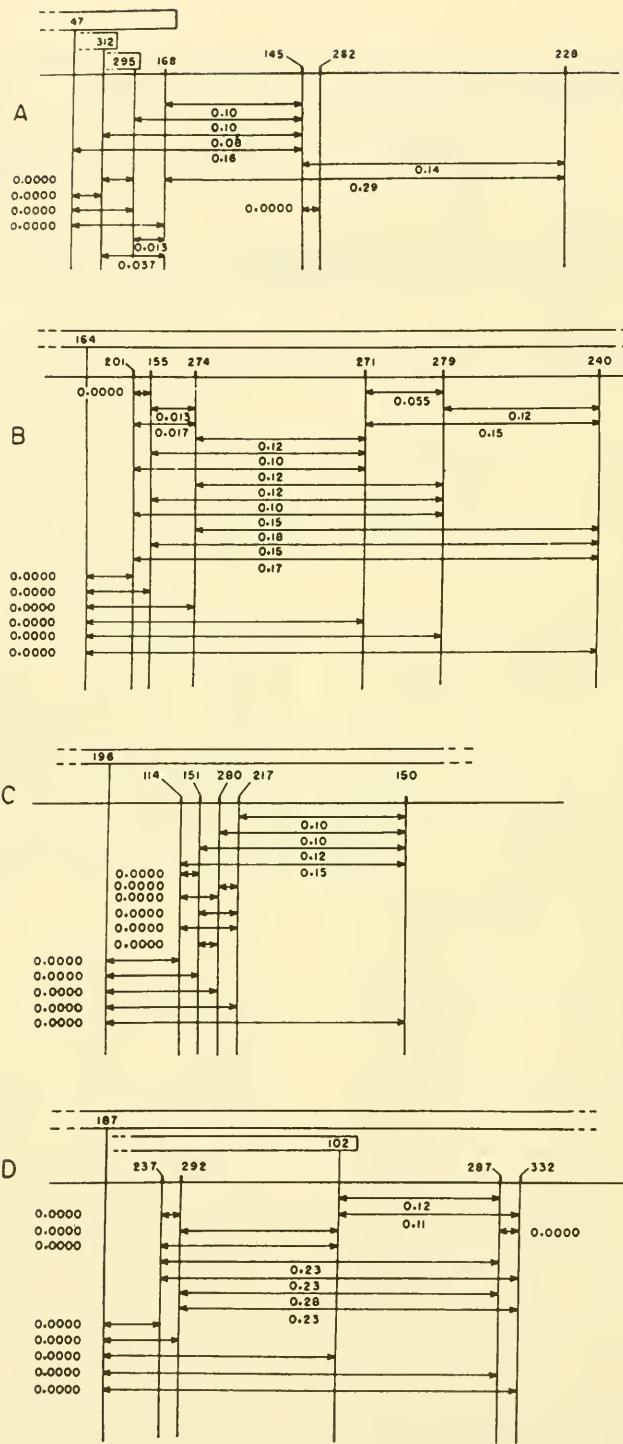


Fig. 5. Maps of microclusters.

region in the wild-type "chromosome" were to control the production of a substance or substances needed for reproduction of this phage in K cells.

The phenotypic test for "pseudallelism" leads to the division of the region into two functionally distinguishable segments. These could be imagined to affect two necessary sequential events or could go to make up a single substance the two parts of which must be unblemished in order for the substance to be fully active. For example, each segment might control the production of a specific polypeptide chain, the two chains later being combined to form an enzyme. While it is not known whether this sort of picture is applicable, a model of this kind is capable of describing the observed properties of the rII mutants. The map position of a mutation would localize a change in the region (and also in the "enzyme" molecule), the reversion rate would characterize the type of change involved in the genetic material, and the degree of phenotypic effect would be an expression of the degree of resultant change in the activity of the enzyme. A "leaky" mutant would be one where this latter effect was small. While no obvious correlation has yet been observed among these three parameters of rII mutants, one may well show up upon more exhaustive study.

"Clustering" of similar mutants separable by crossing-over has been observed for several characters in phage by Doermann and Hill and appears to represent the rule. This may well be the rule in all organisms, simply because functional genetic units are composed of smaller recombinational and mutational elements. One would expect to see this effect more readily in phage because the probability of recombination per unit of hereditary material is much greater than for higher organisms.

By extension of these experiments to still more closely linked mutations, one may hope to characterize, in molecular terms, the sizes of the ultimate units of genetic recombination, mutation, and "function." Our preliminary results suggest that the chromosomal elements separable by recombination are not larger than the order of a dozen nucleotide pairs (as calculated from the smallest non-zero recombination value) and that mutations involve variable lengths which may extend over hundreds of nucleotide pairs.

In order to characterize a unit of "function," it is necessary to define what function is meant. The entire rII region is unitary in the sense that mutations anywhere within the region cause the rII phenotype. On the basis of phenotype tests of *trans* configuration heterozygotes, this region can be subdivided into two functionally separable segments, each of which is estimated to contain of the order of 4×10^3 nucleotide pairs. If one assumes that each segment has the "function" of specifying the sequence of amino acids in a polypeptide chain, then the specification of each individual amino acid can as well be considered a unitary function. It would seem feasible, with this system, to extend genetic studies even to the level of the latter functional elements.

Summary.—It has been discovered that the mutations in the rII region of phage T4 have a characteristic in common which sets them apart from the mutations in all other parts of the map. This characteristic is a host-range reduction, namely, a failure to produce plaques on a host (K) lysogenic for phage λ . The mutant phage particles adsorb to and kill K, but normal lysis and phage release do not occur.

All mutants with this property are located within a sharply defined portion of the phage linkage map. Within that region, however, their locations

are widely scattered. An unambiguous seriation of the mutants, with roughly additive distances, can be accomplished, except for certain anomalous cases.

The simultaneous presence of a wild-type phage particle in K enables the multiplication of rII mutants to proceed, apparently by supplying a function in which the mutant is deficient. A heterozygous diploid in the *trans* configuration is simulated by a mixed infection of K with two mutant types. The application of the phenotype test to pairs of rII mutants leads to the division of the region into two functionally separable segments.

Spontaneous reversion to wild-type had been observed for most of these mutants. It remains to be seen whether these are genuine reversions. Each mutant reverts at a characteristic rate, but the rates for different mutants differ enormously. Partial reversions to intermediate types are also observed.

The mutants differ greatly in degree of residual ability to grow on K. There is no evident correlation between map position, reversion rate, and degree of residual activity of the various mutants.

The selective feature of K for wild-type recombinants offers the possibility of extending the recombination studies to an analysis of the fine details of the region.

Preliminary studies of this type indicate that the units of recombination are not larger than the order of one dozen nucleotide pairs and that mutations may involve various lengths of "chromosome."

I am much indebted to A. D. Hershey and A. H. Doermann for stocks of their genetically mapped mutants, to Sydney Brenner and David Krieg for stimulating discussion, and to Max Delbrück for his invaluable moderating influence.



MENDEL LEUPOLD JOHANNSEN

PUNNETT McCARTY TATUM

WRIGHT STADLER LEDERBERG

CREIGHTON HARDY DUNN

BEADLE WILLIAMS McCLINTOCK

ZINDER MORGAN HOROWITZ

BENZER BRIDGES PAINTER

FRAENKEL-CONRAT CRICK AVERY

SUTTON MacLEOD WATSON

STURTEVANT MULLER BATESON