GENETIC VARIATIONS IN RELATION TO EVOLUTION

H. S. JENNINGS

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*A Critical Inquiry into the Observed
Types of Inherited Variation, in
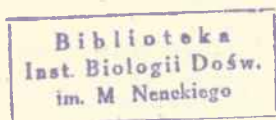Relation to Evolutionary Change*

BY

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FOREWORD

The chapters of this book are based on the materials presented in the Louis Clark Vanuxem Lectures, given in December 1934, at Princeton University.

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CHAPTER I

EXPERIMENTAL genetic science has said of late, with some justification, that "the problem of heredity is solved"; solved in principle, though not in all its details. It *is* solved to the extent that the nature of the processes which underlie it are largely known; and so far as not known, they are localized and open for study.

Can experimental genetic science say that the problem of evolution is likewise in principle solved? Are the processes which underlie evolutionary change known? This question forms the theme of the present study.

The processes which underlie organic evolution are bound to be genetic variations of some sort; that is, changes in the inherited characteristics. Experimental genetic science is now able to detect genetic variations as they occur in living organisms. Single genetic variations are isolated, the precise time and place of their occurrence determined; they are subjected to minute study in the laboratory. Within the last few years men have learned how to produce these genetic variations; this is perhaps the most important condition for determining the nature of such changes. In a thousand laboratories men are at work on these things; genetic variation is now one of the most active subjects in biological science. The observed genetic variations are of a number of diverse types. Do these known types furnish the material of evolution? Are the single variations steps in evolution? Does genetic science therefore

now see evolution occurring, as it sees inheritance occurring? Are the known types of genetic variation adequate to bring about the salient features of organic evolution? These are the questions with which we wish to deal.

What are the salient features of organic evolution? If we could watch the procession of living things from the beginning of life on the earth, what kinds of changes would we see? We can I believe list the main types of change under five heads:

1. First, evolution is transformism. Organisms do not remain constant, but slowly change with the passage of time, as a developing child changes with the passage of time.

2. Second, evolution is diversification. Organisms transform, not in one direction only, but in many directions. From an early simple situation is produced that which we now see about us: great numbers of animals and plants that are diverse in many ways.

3. Third, evolution brings about adaptation. Organisms develop structures and functions that tend to keep them in existence; to ward off dangers in a varied and changing world, and to obtain conditions favorable for their internal processes. Different organisms become adapted to different conditions. Otherwise expressed, evolution produces structures and functions that bring about ends, of the sort that in human beings become the object of conscious purpose. And evolution produces, in man at least, some organisms whose behavior is consciously purposive.

But, what is also important, there are produced, too, organisms that are not adaptive; organisms that fail to develop or to survive under the conditions of existence; a fact to which we shall have to recur. Adaptation is produced in evolution, but is not universal in the products of evolution.

4. Fourth, progressiveness is a salient feature of evolution. Organisms were at first simple; in later ages some of these have transformed into complex highly differentiated animals and plants. From blobs of jelly-like material, responding inadequately to a few of the forces of nature, have developed organisms with elaborate sense organs and organs of response; creatures whose behavior is minutely and adequately correlated with processes in the surrounding world.

But progressiveness, like adaptation, is not a universal feature of evolution. Highly developed organisms may degenerate into parasites, and many organisms still exist that have not developed beyond the lower grades of the organic scale. Whatever brings about evolution must be able to produce progress; but it may produce also its opposite.

5. Fifth and finally, as to the method of progress when progress occurs, we must accept as a fact I believe the reports of students of progressive evolutionary series among fossil organisms such as are known in the development of the horse or the elephants. They report that progress in such series is continuous, gradual, by slight changes, not by sudden leaps. Continuity, not discontinuity, is the characteristic of such progressive series.[1]

Genetic variations, to be adequate to the production of evolution, must then be of a sort to produce these five salient features: transformism, diversification, adaptation, progressiveness and continuity.

And now, certain points as to the nature of genetic variations. In order that variations shall play a part in evolution, they must be inherited; they must affect, not single individuals only but their descendants, becoming thus characteristics of a race or species. Only such can be called genetic variations.

[1] See the notes at the end of each chapter.

There occur great numbers of variations that affect only individuals, having no influence on their descendants. Color becomes changed under action of the sun, but this does not affect the offspring of the individual concerned; and there are thousands of such variations.

Thus for a variation that plays a rôle in evolution—for a genetic variation—two things are essential. There must be a change. And there must be inheritance of the changed condition. A non-genetic variation is like a change in the shape of a crystal, resulting from filing off its angles and edges. Such a crystal, when dissolved and recrystallized will produce anew the original form, for its chemical constitution has not been changed by the alteration in its shape. A genetic variation is like an alteration in the chemical constitution of the crystal, causing it when dissolved and recrystallized, to appear in a new form; a new form that is repeated as often as crystallization occurs without further change in chemical constitution.

Certain other relations play a great rôle in the results of genetic variation. Variations occurring in certain parts of organisms cannot be inherited. To influence later generations they must affect certain particular parts of the body or of the cell. In all higher organisms, reproduction does not occur by mere division of the body of the parent into two equal parts, as may occur in bacteria and protozoa. On the contrary, the new individuals arise from single cells, "germ cells," which are given off by the parents. These germ cells arise from certain well defined parts of the body, the gonads or germ glands. Thus any genetic variations that are to be carried on to later generations must affect the germ cells. If a genetic variation arises in some other part of the body—say in the hand—this cannot be carried on into later generations, for no part of the later generation arises from the hand. Whether

a genetic variation in the hand might in some way cause a genetic variation in the germ cells is another question. But unless the germ cells in some way are affected by the genetic variation, it cannot be carried on to later generations.

Furthermore, even in any single cell, different parts of the living material play very different rôles in heredity and consequently in genetic variation. It is known that the materials that play the chief rôle in determining the characteristics of organisms are those located in the central capsule of the cell —the nucleus. By changing these materials in the nucleus of the fertilized egg—leaving the rest of its materials unchanged —the inherited characteristics of the entire individual produced from the egg are altered. To produce an effect on the inherited characteristics, therefore, a genetic variation must affect these materials. The materials within the nucleus constitute what has been known in the past as the germ plasm; genetic variations are changes in the constitution of the germ plasm.

Until recently very little was known of the germ plasm; its existence was assumed but speculative. But it has now been identified, studied minutely, experimented on in many ways. It is found to have an elaborate organization. On the nature of this organization depend in large measure the kind of genetic variations that occur, and their effect in evolution. The first requirement therefore for understanding genetic variation is to have before one a picture of this organization. It is to such a picture that we first turn our attention.

An organism, as we know, begins its life as a single cell, which we call the fertilized egg. The germ plasm, located within the nucleus of this cell, is constituted of a large number of diverse materials, which we may call genic materials. Each type of genic material is concentrated into a small particle, commonly known as a gene. The entire germ plasm

thus exists in the nucleus as a large number of separable particles or genes, grouped into the chromosomes.[2]

These genic materials are extremely active in the life of the organism. From within the nucleus the genes send out products of their action into the remainder of the cell—the cytoplasm. Here the genic products interact with each other and with the cytoplasm itself, so becoming modified in many ways, and producing many different things. In this way they produce the various tissues of the body, and give rise to its structural and physiological peculiarities; to what we call its characteristics.

But of each of these genic materials there is retained an unmodified part, a reserve portion, enclosed within the nucleus. It is these unmodified reserves that are called the genes. When cell division occurs, each of these unmodified reserves, or genes, divides, so that each of the cells produced receives a half of each gene; this grows again to form an entire gene. Thus every cell of the body has the entire stock of reserve materials or genes.

Genic materials therefore have two most important properties. First, they affect development, and thus help to determine the characteristics of the individual produced. And second, each genic material assimilates, grows, and reproduces true to type; each gene, in other words, produces additional material of the same kind as itself.

Half of the genic materials within the nucleus come to the fertilized egg from one of the two parents, half from the other parent. They are built up in the nucleus into a complex structural system, just as the nervous material is built up into a complex nervous system. This system of genic materials we may conveniently call the *genetic system*. Its peculiarities play a great rôle in determining the nature of genetic variations, so that we must examine them.

The small particles into which the genic materials are concentrated—that is, the genes—are strung up in series, like beads on a string. All those that come from the father form one series or string; all those from the mother form another series, another string of beads. Thus there are in the cell two strings of genes. These constitute what I am calling the Genetic System. This constitution of the genetic system is shown as it appears under the microscope in Figure 1, while Figure 2 shows a diagram of its chief features.

The system is made a little more complex, but in a relatively unimportant way, by the fact that each string, paternal or maternal, is broken into a number of segments, which in their condensed conditions are commonly called chromosomes (D, Figure 1). Essentially, however, there is one string of genes from the mother, one from the father, so that for the present we may neglect the segments into which each is broken. The different genes in either of the two strings are diverse organic chemicals, with different properties, different effects on the development.

Each gene, each different kind of material, has its regular place in the string. Thus the different genes can be named or numbered. Gene No. 1 has a certain definite effect on development; gene No. 40 another effect; gene No. 200 another, and so on for all of them. Thus maps showing the relative positions of the different genes can be made; they have been made for certain organisms that have been thoroughly studied. Changing a single one of the genes—altering it, or substituting for it a different one—changes the course of development, and causes the adult organism to have changed characteristics. Altering one of the genes changes the color of the eye; altering another changes the size or stature; altering another changes the structure of a limb; altering another changes certain physiological processes. Every feature of the organism,
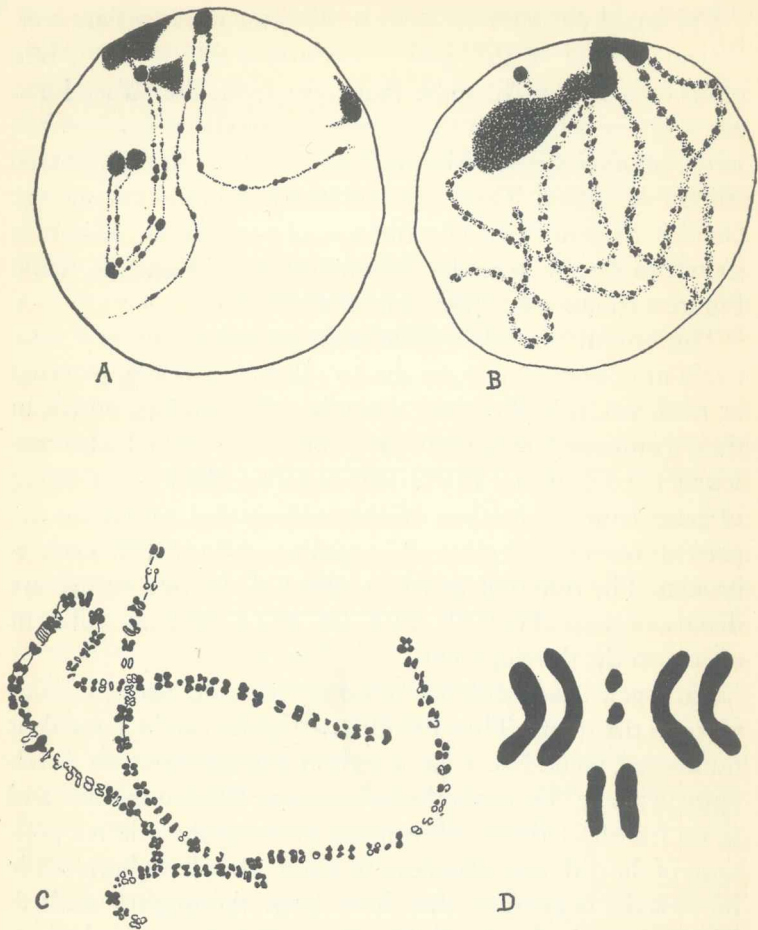
Figure 1. Characteristic features in the organization of the germ
plasm. The chromosomes and chromomeres, as seen under the micro-
scope, in different developmental stages. *A* and *B*. Chromosomes and
chromomeres in nuclei of the grasshopper, in the thread-like stage of
development, after Wenrich, 1916. The enlargements on the threads,
known as chromomeres, probably show the location of the different
genes: in any case their arrangement and position are the same as

that known for the genes. *C.* Much enlarged chromosomal threads of the lily, showing the chromomeres (genes?), after Belling, 1928. *D.* The chromosomes in the condensed condition (chromomeres not visible), in the female fruit fly, Drosophila melanogaster, after Metz, 1916.

structural or physiological, may be changed by altering one or more of the genes; by substituting one gene for another. The entire practice of genetic science, of animal breeding, is an elaborate technique for substituting one gene for another and determining the consequences of such substitution.

Different individuals are found to have genes that differ from each other in some of their properties; in consequence of this, different individuals have different characteristics. To this fact are due most of the diversities between individuals; differences in features, in body form, in size and in structure; differences in physiology; differences in senses and behavior; differences in character and personality. With increase of knowledge it has become more and more clear that most differences of all kinds between individuals are due to differences in the materials of which they are made; that is, in the genes that they carry—rather than to differences in the conditions under which they have lived.

When a new individual is formed—constituting at the beginning a new fertilized egg—this new individual gets half his genes from his mother, half from his father. And now a most important fact! The genes that he gets from one parent —as from the mother—form a complete series, containing genes for every function in development. This series contains all the genes that are necessary for development and for producing all the structures and functions of the body.

And the other chain of genes, that he gets from his other parent, likewise contains all the genes that are necessary for
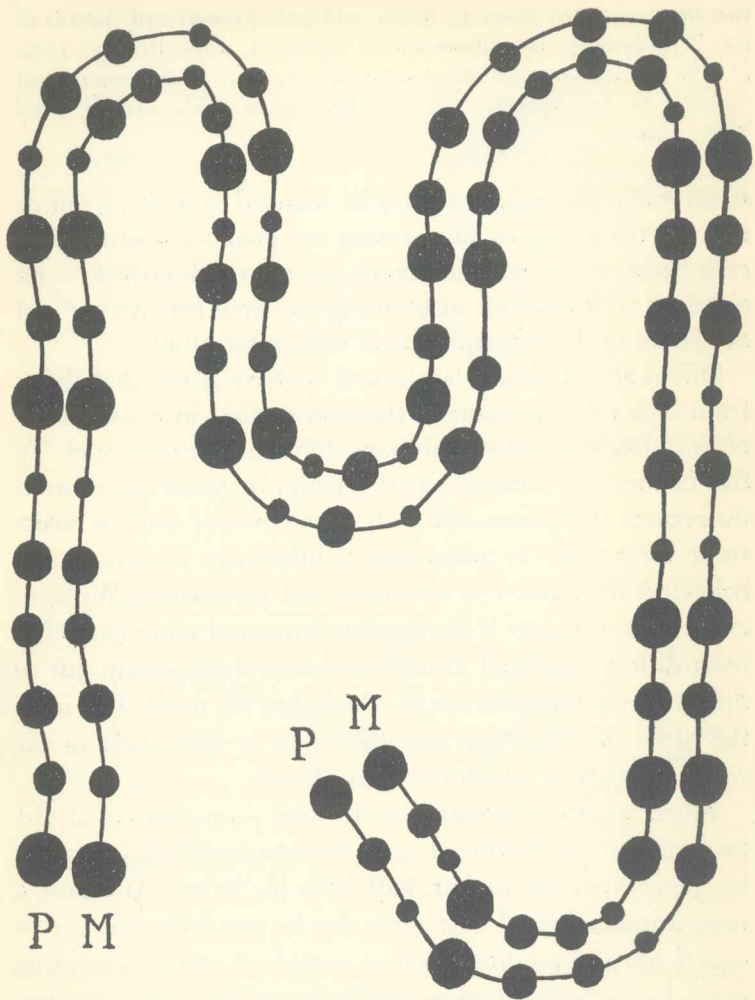
Figure 2. Diagram of the organization of the germ plasm. Two chains of genes, one (*P*) from the father, one (*M*) from the mother; so that the genes are in pairs, one member of each pair from each parent.

the development of a complete individual with all its struc-
tures and functions. That is, the new individual is double as
to his genes; he carries two complete sets,[3] each containing all
that is necessary for producing an individual. For every gene
in the maternal string there is a corresponding gene in the
paternal string (there are certain exceptions in some organ-
isms), so that the two strings are throughout parallel in
structure and action. Thus every kind of gene is represented
twice—by one example from the mother, the other from the
father. So the genes are in pairs—one gene maternal in origin,
the other paternal in origin. Each function, in development,
each effect, has two genes, either of which may perform it.
This doubleness of organisms in respect to their genes is one
of their most important features; it must never be forgotten.

But the two genes of a pair, though they have in general the
same function, may, and often do, perform that function in
a different manner. One of them, from the mother, tends to
produce eyes of a certain color—in man, for example, brown
eyes; while the one from the other parent may tend to pro-
duce eyes of another color—blue eyes, perhaps. One of a pair
may tend to produce a rapidly growing, vigorous body, the
other a slow-growing, weak body. One of a pair may tend to
produce acute hearing, the other defective hearing. One may
tend to produce blood of a certain type of the several possible
blood types, the other another blood type. And so of all their
effects.

In such cases, where the two corresponding genes differ,
usually one of the two prevails over the other. If one tends to
produce brown eyes, the other blue, the brown-eye gene pre-
vails and the individual has brown eyes; these have more
pigment than blue. If with respect to any function or struc-
ture one gene tends to produce a vigorous or normal individ-
ual, the other a weak or defective one, the one that tends to

produce the vigorous or normal condition usually prevails over the other; the individual is not weak or defective. In general, it may perhaps be said that the gene that carries development farthest usually prevails over the other, though whether this is the invariable rule is doubtful. The gene that prevails is said to be dominant; the one that does not prevail, that produces no obvious effect, is said to be recessive. It is only when two of the weak or recessive genes get together in a pair—one such coming from each of the two parents—that the weak or defective or recessive characteristic is produced and appears in the individual.

Thus each individual is double with respect to his genes. Each contains a number of genes that have no manifest effect; a lot of recessive genes, whose effect is suppressed by their dominant companion genes in the same pairs. Thus one gene of a pair can become modified, become defective perhaps, without producing any obvious effect on the characteristics. But if two parents each contain such a defective gene, in the same pair, then when they mate, some of their offspring may get a pair of genes in which both members are recessive —weak or defective—and these offspring will manifest the weak or defective characteristics, though their parents did not.

Such then is the picture of the germ plasm, or of what we may call the genetic system, a picture that we must keep in mind in trying to understand genetic variation. In any individual a double set of genes, frequently only one of each pair of genes manifested, because dominant; the other recessive, producing no effect (see Figure 2).

If we examine different individuals we find that the genes of a certain pair—having a certain general function—may exist in many different modifications in the different individuals. So a certain gene pair, located at a particular point in the chain, that affects the eye color in the fruit fly, is known

to exist in at least a dozen different modifications, in different individuals. One of the modifications produces red eyes, another white eyes, another buff eyes, and others produce various shades of red, or mixtures of red and yellow, or slightly tinged, or ivory eyes. Similar modifications are known for many other genes, each producing a slightly different effect; they probably occur in all the genes.[4] In this way, by diverse modifications of the same gene, a great number of different types of characteristics are produced; different eye colors, different forms, different sizes, different degrees of strength and vigor, different physiological activities. Every structure and function of the organism, without exception, is thus capable of being produced in different grades and qualities, through diversities among the genes.

If we look at any species of animal or plant as a unit, including all the different individuals that compose it, we find then the following situation as to genes: First, there is a great number of different types of genes, strung up in a long series. Each type has its special function, and each has its definite place in the series; it is No. 1 or No. 40 or No. 200, or the like. Any individual has two genes of each of the types. But, second, each type has a number of different modifications, each modification performing the given function in a somewhat different way. If the gene type is one that affects the color of the eye, then the various modifications of that type give in different individuals a whole series of different eye colors. Since each individual has just two genes of a certain type, a single individual can contain only two of the modifications of any type; other individuals may contain other modifications.

Thus what characteristics are shown by any particular individual depends on what combinations of the various types and modifications of the genes are present in his cells. And

since the number of types and modifications is very great, the number of diverse combinations of characteristics that may occur is practically unlimited.

Observe now the relation of all this to genetic variations. The genetic system presents three diverse aspects, each of which may be altered; each of the three yields a distinctive type of genetic variation. First, the genetic system is composed of many diverse organic materials, the genes. These materials might be altered, chemically or otherwise, giving rise to new characteristics; this would be the fundamental type of genetic variation. The genes are arranged in a definite structure, the genetic system. This structure may be altered; this gives a second type of genetic variation. Third, within the framework of this structure, there are diverse combinations of genes, in different individuals. Changing the combinations of genes that are present gives a third type of genetic variation.

Genetic variations of all these types are known, or at least are commonly held to be known; they have been described in detail. About one of the three types, however, serious doubt has recently arisen; this is a matter with which we shall deal later (see Chapter III).

We shall take up these three types of genetic variation in the reverse order from that in which I have named them. We shall take up first, changes in the combinations of genes present; then changes in the structure of the genetic system, reserving the supposed changes in the material of the genes for our third topic. Beyond this I plan to present certain other genetic variations not precisely fitting into this classification. In the limits of these pages I shall have to confine myself to types of variation *observed* to occur in *living* organisms. We shall look at each type in relation to evolution.

GENETIC VARIATIONS RESULTING FROM NEW COMBINATIONS
OF GENES

There is in nature a great mechanism, always operating, for the production of new combinations of genes, and thereby giving rise to genetic variation on a grand scale. The operation of this mechanism is what we call biparental reproduction, with its consequences that we call Mendelian inheritance. This is perhaps the most potent means that is known to us for yielding genetic variation and promoting evolutionary advance. Its operation therefore we must sketch.

Heredity is commonly thought of as a matter of resemblance, rather than of diversity; resemblance between related individuals. But Mendelian heredity produces diversity as well as resemblance. Production of diversity is indeed perhaps its most conspicuous feature; it might equally well be called Mendelian variability. From a single pair of parents there arise by Mendelian inheritance offspring of many diverse types. Any two unrelated human parents doubtless have the capability of producing offspring of a thousand diverse types. Certain features of this process are of great interest for the problem of genetic variation and evolution.

Each individual, as we have seen, has a certain combination of genes, some dominant, some recessive; half of them have come from his mother, half from his father. This definite combination of genes results in producing, under the given life conditions, a certain set of characteristics, structural and physiological. They give a body of a certain size, form and structure, with functions that are performed in a certain way; with a certain type of mental characteristics and behavior. They give an individual with a definite personality.

Now, when individuals reproduce, this combination of genes is taken apart, and a new one is made by putting to-
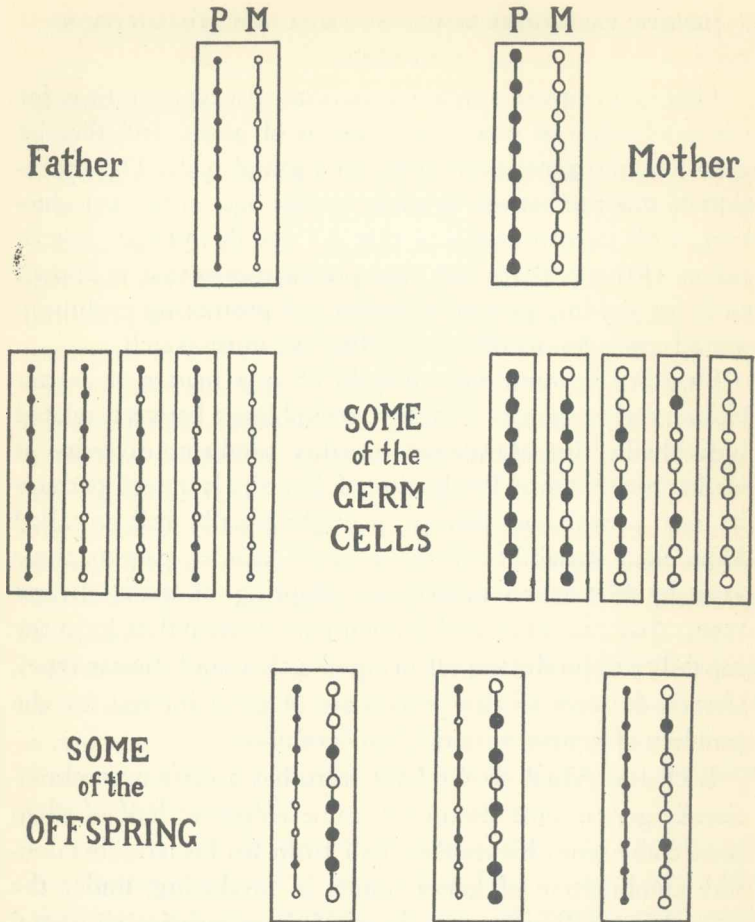
Figure 3. Diagram to illustrate the recombination of genes when offspring are produced. Above are shown, in the two rectangles, a small portion of the double string of genes in each parent; in each string but eight of the many genes are shown. In each parent the genes of paternal origin (*P*) are represented as solid, those of maternal origin (*M*) in outline. In the middle row are shown a few of the many possible different combinations of genes in the germ cells, pro-

duced by taking one gene of each pair for each germ cell. In the lower row the three rectangles show three of the great number of diverse combinations producible by union of one germ cell from the father, one from the mother.

gether half of the genes from each of the two parents. This gives a combination of genes that is different from that of either of the two parents; so that the child will have a different set of characteristics from either parent. There may thus be produced a combination of genes never before produced, giving an individual with characteristics different from any that have before existed. To this method of operation is due a great deal of the diversity that exists in nature. To it is due at least a certain amount of what we may call evolutionary progress; just how much it is difficult to say in the present state of our knowledge. Examination of some of the rules of this method of producing new combinations will help in grasping it.

The great rule for the production of a new individual by two parents is this: Each parent gives to the child *one* member of each of his own pairs of genes. This is indicated in the diagram of Figure 3. If the parent has a thousand pairs of genes, he gives to each child one member of each; a thousand single genes in all. The other parent gives also one member of each of his thousand pairs, so that the child gets again from both together a thousand pairs.

Each parent may give to the child either member of any of his pairs of genes. We may designate the two members of any of the parent's pairs as maternal (M) or paternal (P), according as they have come to him from his mother or his father. The child may get then either the maternal gene or the paternal gene. Frequently, perhaps usually, these two members of the pair are different in their effects. Commonly one
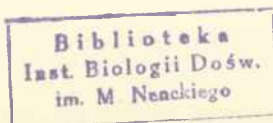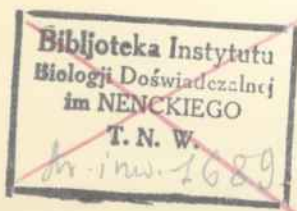
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is dominant, one recessive. The two members may differ (in diverse cases) in many gradations of effect, or in many qualities. One may tend to produce blue eyes, one brown eyes. One may tend to produce vigor, the other weakness; and similarly for all structures and functions. The child gets one of the two, not the other; he may get either. Some of the offspring get one of the two, some get the other.

Furthermore, the different pairs of genes of the same parent are in a measure independent in the way they are distributed to the offspring. If the child gets the paternal gene of one pair, he may get either the maternal or the paternal gene of any other pair.[5] Thus there is a great number of possible different combinations of genes from one parent that may pass to diverse children. If there were but two pairs of genes there would be four diverse combinations, any one of which might be received by the offspring. For four pairs the number of different combinations is 16; for ten pairs it is over a thousand; for one hundred pairs the number is unimaginably great (it is two to the hundredth power). Since for higher organisms the number of pairs of genes is much above one hundred, the number of different combinations of genes that may be received by the different children from one parent is very great.

From the other parent the diverse children may receive an equally great number of different combinations of genes. Any combination from one parent may meet in the child any combination from the other. The result is that the number of different types of offspring that might be produced from a single pair of parents is almost inexpressibly great. Each of these types of offspring would have a different set of genes and a different set of characteristics.

Thus reproduction from two parents is a great process of producing individuals with new combinations of genes and

new combinations of characteristics. Every combination of genes that is mathematically conceivable may thus be produced. Every possible combination of the characteristics that depend on the genes may thus be brought into existence. To take a simple example, if one parent has blue eyes and light complexion, the other dark eyes and dark complexion, then among their descendants will be some like the fair parent, some like the dark parent, some with blue eyes and dark complexion, some with dark eyes and light complexion. If the parents differ in many characteristics instead of but two, the number of different combinations of characteristics among their descendants is very great.

In this production of new combinations through reproduction from two parents, there are three types of consequences that are of interest for evolution.

First, in this way there are produced combinations of genes, and therefore combinations of characteristics, that have never before existed.

Second, in this way single qualities, powers or dimensions, may be increased beyond the degree in which they have before existed.

Third, as a result of the recombinations of genes, characteristics may appear in the descendants that have never occurred in their ancestors, or in any other organism.

In these three ways therefore, evolutionary change is brought about by reproduction from two parents. We will consider each of the three types of change separately.

1. By biparental reproduction, any quality or peculiarity of any individual or any stock may become combined with any features of any other individual or stock with which it can unite for parenthood. Thus are produced combinations of characteristics that have never before been brought together. This plays a great rôle in relation to the fitness or adaptiveness

of organisms. The result may be individuals superior in some respects to any that have before existed, so that a distinct evolutionary advance has been made. One stock may have strength and agility, but also poor sight and hearing. Another may have acute senses, but be weak and slow. By the mating of the two, some offspring may be produced that have all the superior qualities combined: strength, agility and acute senses. Such combinations have a great advantage over either of the parent stocks; a superior type has been produced. Superior combinations are thus produced in thousands of different ways, by the mating of diverse stocks.

Of course inferior combinations are produced too in the same manner. By mating the stock that is strong and agile but has poor sight and hearing, with the one that is weak and slow but has acute senses, some combinations are produced with all the inferior qualities; they are weak, slow, and have poor senses. Such individuals are not successful in the struggle of life; they soon disappear, leaving only the better combinations. Along with the production of new combinations by biparental reproduction, there is selective elimination on a large scale, getting rid of the poor combinations, retaining those that are efficient. Thus through the formation of new combinations, along with selective elimination, progress is brought about.

2. The second effect of biparental reproduction is the strengthening or increase of single powers and qualities, beyond the previously existing degree. It is known that size, strength, vigor, acuteness of senses, and other faculties or characteristics that play an important rôle in life, do not depend on single genes only; each is known to be influenced by many diverse genes. Some of these genes are of superior type in some individuals, others are superior in other individuals. Thus one individual or race may carry a certain number

of genes that tend to produce vigor and mental power. Another may carry another and different set of genes that produce a degree of vigor and power. When these two are mated, some of the descendants receive both sets of genes that give vigor and mental power; they show therefore a vigor and power far beyond that of their parents. By successive matings of this sort, any particular capability or quality may be carried far beyond the degree in which it formerly existed. What may be the limits of progressive evolution occurring in this way no one can say.

3. Third and perhaps still more important, as a result of these recombinations of genes, characteristics may appear in the descendants that have never occurred in their ancestors. Examples of such results are abundant in animals and plants. They are perhaps most obvious in relation to colors. To take a well known example, we may begin with two races of primroses, one red, the other white.[6] Crossing the two, we find in their descendants individuals having many other colors: magenta, pink, many different shades of red, and flowers with colors in blotches. In place of two colors we have in the descendants a dozen or more different colors. These are obviously due to the chemical interaction of the materials derived from the genes of the two parent races. The different descendants get different combinations of the genes of the two parents. In development these genes give off materials into the cytoplasm. There they interact, producing many different results in different cases. There are known great numbers of examples of the production of new colors, and also of new forms, in this way. The same method of action must apply to other characteristics that are less conspicuous and less readily defined; they must apply to vigor, strength, special capabilities, and mental power. How far

such interaction of gene products may go in producing new characteristics, not before seen, no one can say.

All these three methods of operation occur in man as well as in other organisms. They occur in relation to mental and temperamental qualities as well as to others. One parent may be industrious and ambitious, but fail in life because he is mentally dull and slow. The other may be quick and acute mentally, but fail because he lacks industry and ambition. Among their descendants would be some that combine the industry and ambition of one of the parents with the acuteness and quickness of the other. Such descendants would be superior. By fusing thus all the best qualities of numbers of different ancestors, persons far beyond the usual level would be produced. Again, as we have seen, existing powers and capabilities may in this manner be increased in degree, and even qualities produced that did not before exist. Such methods of operation are doubtless the origin of the individuals that are superior to most of humanity, the geniuses, the Goethes and Leonardos.

Thus this method of operation by the formation of new combinations through the mating of two individuals is powerful in producing variation. A very large proportion of the variety found among organisms is due to it. If reproduction occurred only by the division of single individuals, as may occur in plants and many lower animals, the world of living things would be relatively simple; there would be comparatively few living types. The variety among organisms is multiplied a thousandfold—perhaps a millionfold—by reproduction from two parents instead of from one.

And reproduction from two parents has, as we have seen, great potentialities for the production of new types, and so for bringing about progressive evolution. It is certainly one of the greatest factors in evolutionary progress.

But for the production of new types that are permanent, that bequeath their peculiarities to their descendants, this method of operation suffers under a very great limitation. This is because the interaction that produces the new characteristics occurs between the products of the genes in the cytoplasm, not between the genes themselves. When reproduction occurs, all these cytoplasmic gene products are lost with the death of the body; only the unaltered reserves, only the unmixed genes themselves, go into the germ cells that produce the new generation. And further, in reproduction from two parents, the combination of genes that gave origin to the new characteristics is taken apart and the genes are recombined in new ways, in the next generation. Thus the new types, with their perhaps superior characteristics, are destroyed as types, through the same process that produced them. The new or superior types so produced are taken apart at each reproduction; their components are scattered and combined again to form other types. No type produced by recombination of genes is permanent, so long as reproduction from two parents occurs in every generation.

But in many organisms, including the higher plants and many lower animals, reproduction from two parents is not the invariable method. Reproduction may take place by the division of single individuals. This gives a method of perpetuating the new characteristics and new combinations that have been produced in the way we have described. In such reproduction from a single parent, the existing combination of genes is not taken apart. Each gene divides, half of it passing into the cells of each of the new individuals. All the individuals produced by this method of uniparental reproduction have therefore the same combination of genes; a combination identical with that in the original parent. A great number, an entire stock or race, is produced, all having

the same combination of genes, all having the same hereditary characteristics.

It is through this method that is produced much of the astonishing variety seen in cultivated plants. This is the reason why the horticulturist is able to progress so much more rapidly than the animal breeder in developing new types. By mating together different strains he produces a great number of diverse combinations, some of them differing very greatly from the original stocks. These combinations he multiplies and makes permanent through reproduction from the single parents—by cuttings, bulbs, and similar methods.

But this method cannot be used with the higher animals and man. Here reproduction from two parents is the only method. Every combination produced in one generation is taken apart in the next. A slow and inefficient substitute for uniparental reproduction in making the new combinations permanent is found in inbreeding—the mating of close relatives. Individuals having the same, or nearly the same combinations of genes, are mated. Their offspring necessarily differ much less from their parents and from one another in their combinations of genes than in the usual case of mating between parents with very diverse sets of genes. By continuing for many generations the mating of close relatives, stocks may be produced that are nearly uniform and permanent—until they are again crossed with other stocks. In this way even in the higher animals stocks are producible that are lasting, and that differ greatly from the stocks from which they took origin. By selecting and isolating the best of these, great improvement can be brought about. Some time, some ruthless race, like the Japanese, will apply this procedure to man, with possibly startling results.

Biparental reproduction and inheritance thus plays an important rôle in evolution. It brings about transformism, it

brings about diversification. There being given a great variety of different types of genes, it brings about adaptation and progress—through the method of selective survival and elimination. But it requires the previous existence of many diverse types of genes, and it does not produce these diverse types. What it can do is limited by the potentialities of the different genes already existing. These as yet unrealized potentialities of the genes now existing are doubtless very great, and these may in the future be brought to realization by the making of new combinations among them. But the actual production of diverse types of genic materials is required; it is the essential feature of evolution. To understand evolutionary progress, the method of producing new types of genic materials must be sought.

To this search for the fundamental factor in evolution much effort has been devoted. The sought-for changes in the nature of genes have been called mutation; and many discoveries of mutation have been announced. But often the announcements have been later discovered to be errors; the changes observed were something much less fundamental. This is a history which at the present moment appears to be repeating itself on a grand scale.

But in this search, other types of genetic variation, of only less importance, have been discovered. Great numbers of genetic variations have been found to be the result of irregularities in the structure and operation of the genetic system. These things we examine in the next chapter.

NOTES ON CHAPTER I

[1] Page 3. See the discussion of this matter in H. F. Osborn, "Aristogenesis, the Creative Principle in the Origin of Species," *American Naturalist,* Vol. 68, 1934, pp. 193-235.

[2] Page 6. For detailed accounts of the constitution of the germ plasm—the chromosomes and genes—see some modern text-book of cytology; for example, L. W. Sharp's *Introduction to Cytology* (New York, 1934), or E. B. Wilson's *The Cell in Development and Inheritance* (New York, 1928). For details as to their relation to genetics, some detailed text-book on that subject may be consulted, such as the author's *Genetics* (New York, 1935), or Sinnott and Dunn's *Principles of Genetics* (New York, 1932). A simplified account of both of these matters will be found in the present author's *The Biological Basis of Human Nature* (New York, 1930).

[3] Page 11. Practically all statements in biology are subject to exceptions. A certain limited number of needed genes may be lacking in the set supplied by one of the parents.

[4] Page 13. As will be shown in Chapter III, some or all of these "modifications" of a particular gene may possibly be simply the same gene in different relations to other genes, and yielding different effects on account of these different relations. For the present the text follows the account commonly given.

[5] Page 18. There is a tendency for two genes that are close together in the maternal series or in the paternal series to remain together in the offspring. But this is not fixed or absolute; genes that are side by side may separate into different offspring.

[6] Page 21. Description and plate on this in Wm. Bateson, *Mendel's Principles of Heredity,* 1909, p. 294 and Plate VI.

CHAPTER II

(1) Genetic Variations Resulting from Irregularities in the Structure and Operation of the Genetic System
(2) Gene Mutations

IN SEARCHING for the method by which new kinds of genes are produced, investigators have come upon another type of genetic variation. The genetic system is a mechanism, operating in a complicated fashion. As the cells divide and other operations occur, the genetic system appears under the microscope to be of an astonishingly machine-like character. And like other mechanisms it is subject to getting out of order, to accidents, to breaks and irregularities of operation. When it fails thus to act in the expected manner, it causes changes in the characteristics of the individuals that are produced; it yields genetic variations.[1]

This production of genetic variations by breaks and irregularities in the genetic system plays at present a great rôle in genetic science, and it seems destined to play a still greater rôle in the future; one till recently unsuspected.

As you recall, the different materials of the genetic system—the genes—are strung up in long strings (Figures 1 and 2). These strings are divided into segments, which in their condensed condition we call the chromosomes. Since a set of genes comes to the individual from each of his two parents, there are two sets of genes, and therefore two sets of chromosomes in his cells. The chromosomes, like the genes of which they are composed, are therefore in pairs; one member of each pair derived from the individual's father, one from his mother.

When the individual himself forms germ cells, the two chromosomes of any pair separate into different germ cells; the maternal member of the pair going to one germ cell, the paternal one to another germ cell (Figure 4). Thus each germ cell receives but one chromosome of each pair (and consequently but one gene of each pair). Later two germ cells (sperm and ovum) unite, and as each brings a member of each pair of chromosomes, the new individual or fertilized egg again has its chromosomes (and genes) in pairs, as shown in Figure 4.

Parents   Germ Cells   Fertilized Egg

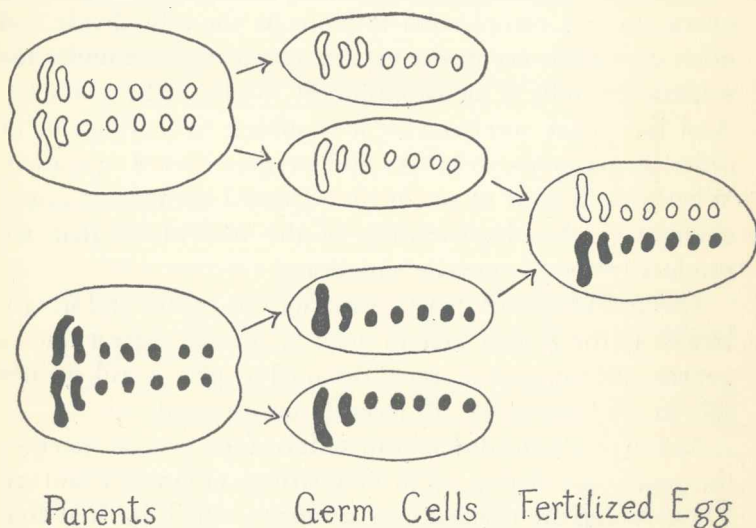*Figure 4.* Diagram of formation of germ cells and their union to form the fertilized egg, to show the separation of the chromosomes of each pair into different germ cells, and the re-formation of the pairs in the fertilized egg.

We come now to the accidents and aberrations that give rise to genetic variations. The complex and delicate operations that we have just described do not always take place accu-

rately or according to specifications. In rare cases, in forming
germ cells, the two members of a pair of chromosomes fail to
separate (Figure 5, *B*). Then one germ cell is formed that has
two members of this pair, while another has no member at
all of this pair. This gives rise to many further irregularities.

Parents   Germ Cells   Fertilized Eggs
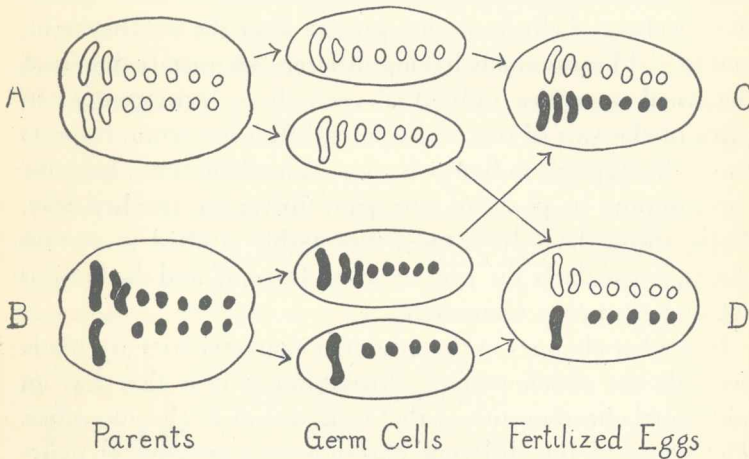
Figure 5. Diagram showing how the number of chromosomes some-
times becomes changed through irregularities of division. The cell *A*
divides in the usual way, producing germ cells containing one chromo-
some from each of the seven pairs. In the division of the cell *B*, both
members of the second pair go to one of the germ cells, which thus has
eight chromosomes, while the other germ cell has no chromosome of
the second pair. By union of the normal germ cells from *A* with the
changed germ cells from *B*, as indicated by the arrows, a germ cell *C*
is produced that has three chromosomes of the second pair, and
another germ cell *D* with only one chromosome of the second pair.

When the germ cell with two chromosomes of a pair unites
at fertilization with one of the usual germ cells, an individual
is formed that has three chromosomes instead of the usual
two (Figure 5, *C*), for this pair; the genes of this pair are

then in threes instead of in twos. And when the germ cell with no chromosome of this pair unites with a typical germ cell, an individual is formed with only one chromosome of that pair; its genes are single instead of in pairs (Figure 5, *D*).

Many cases of this sort have been discovered, in many different animals and plants. It is found that such changes in the numbers of chromosomes present alter the development, and so yield individuals having different characteristics from the usual ones. An individual with three chromosomes in place of the two of one of its pairs differs in certain respects from the typical individuals; an individual with but one chromosome in place of one pair differs in another way. These things have been most thoroughly studied in certain plants, particularly the Jimson weed, Datura,[2] and the famous evening primrose, Oenothera.

Just what changes in the inherited characteristics are made depends on which pair of chromosomes it is that has an additional chromosome, or that lacks one of its chromosomes. The genes of the different chromosomes are diverse materials, with different functions, different effects on characteristics. Hence adding a chromosome to one pair has a very different effect from adding it to another pair; and the same is true of subtracting a chromosome from a pair. Since any organism has, as a rule, several or many different pairs of chromosomes, a large number of different heritable types can be produced in such ways.

An example will give an idea of the variety introduced into organisms in this way. In Datura, the Jimson weed, there are twelve pairs of chromosomes. Adding a third chromosome to any one of the pairs gives in each case different characteristics. Hence there are twelve different varieties producible through adding a chromosome in different cases to the twelve different pairs. Also there are twelve other varieties formed by taking

away one chromosome in different cases from each of the twelve different pairs. Many of these twenty-four different varieties have been produced and studied by Blakeslee and his associates.

In a similar way, two of the pairs may have a third member added to them. For twelve chromosomes, there are sixty-six different ways in which this can be done. This gives sixty-six other differing varieties, many of which have been produced and identified. And there are sixty-six other varieties producible by subtracting one chromosome from two pairs, in the sixty-six possible combinations of two from twelve.

When individuals with three chromosomes in place of one of the pairs produces germ cells, some of these germ cells have two chromosomes of that pair, others but one (see Figure 6). When two germ cells unite, each con-
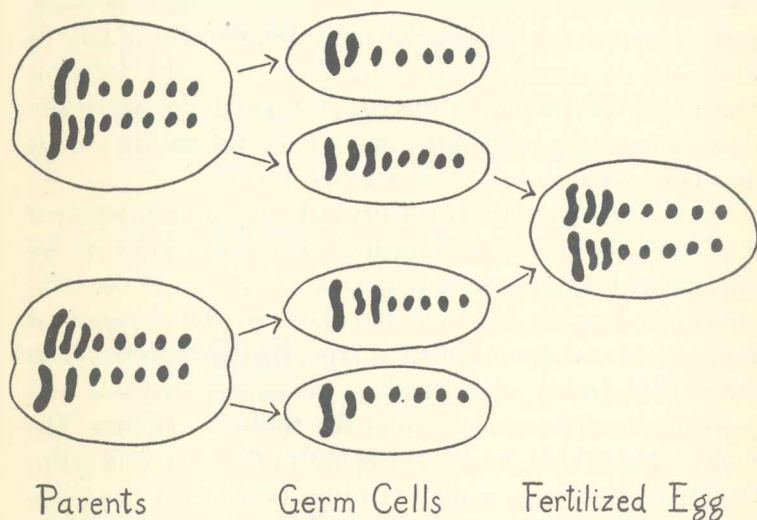

Parents   Germ Cells   Fertilized Egg

Figure 6. Diagram of the method by which there are produced individuals with four chromosomes in place of one of the pairs (in this case the second pair, counting from the left).

taining two chromosomes of that pair, we get an individual that has four chromosomes belonging to one of its pairs; the genes of these chromosomes are thus in fours. Such individuals have different characteristics from any of the others of which we have spoken. There are in the Jimson weed twelve different varieties obtainable by giving four chromosomes in place of two, in different cases, to each of the twelve chromosome pairs. And sixty-six more types can be formed by giving four chromosomes to each of two of the pairs, in each of the sixty-six different combinations of two from twelve.

Thus the number of diverse types of organisms producible in these ways is becoming large. We have thus far enumerated two hundred and thirty-four possible types. It might appear that we could go on multiplying varieties indefinitely in this way, by adding or subtracting one or two, to each of three pairs, then to each of four pairs and so on. But nature fails us when this is attempted. If more than two pairs are thus changed by addition or subtraction, yet not all the twelve are thus changed, the individual cannot live; it seems then to be unbalanced and so incapable of development.

Yet if all the chromosome pairs lack one chromosome—or if all have one or two additional chromosomes—then all being changed equally, there is no lack of balance, so the individuals do not die, but live and develop. Our diagram of Figure 7 shows various different types that are producible in these ways. In the diagram there are represented but four pairs of chromosomes instead of the twelve of Datura. The normal individuals, known as diploids (B), have four pairs. Others are known in many organisms in which the chromosomes are single instead of paired; these are known as haploids. Others have three chromosomes of each kind (in place of but two or one); others have four of each kind (tetra-
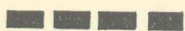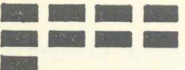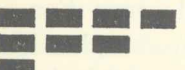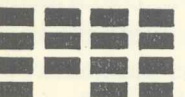
A	Haploid	n = 4
B	Diploid	2n = 8
C	Triploid	3n = 12
D	Tetraploid	4n = 16
E		2n-1=7
F	Trisomic	2n+1=9
G	Double Trisomic	2n+2=10
H		2n+1-1=8
I		4n-1=15

Figure 7. Diagrams showing some of the different groupings of chromosomes, that may produce living organisms having different hereditary characteristics. In this case the normal grouping is shown at *B*; four pairs of chromosomes. At *A* the chromosomes are single instead of in pairs, at *C* they are in threes, at *D* in fours. In *E* to *I* are various other groupings such as have been observed in some cases.

ploids). All these different types live and develop; they are known in many organisms. Figure 7 represents also other types that live: those in which one or two pairs have an additional chromosome, and the like.

By adding or subtracting one or two chromosomes to the different groups ("pairs") in these tetraploids or other types, and in other ways, a great number of varieties are producible from a single type. Blakeslee and his associates have produced and studied in this way eighty-nine different varieties in Datura, the Jimson weed. And these are but a fraction of those that are possible. Computations show that in Datura there must be three thousand six hundred and twenty different possible types producible from the single typical variety, by thus altering the grouping of the chromosomes and genes. There is reason to believe that all of these can be produced and that they will live.

The conditions thus carefully studied in Datura are not exceptional; they are found in many other organisms, particularly in plants. They have been very extensively studied also in Oenothera, the evening primrose. Here again there are many different varieties or types, resulting from changes in the grouping of the chromosomes and genes. You will remember that it was on the evening primrose that Hugo De Vries made the famous studies that he published about the year 1900 in the great two-volume work, *Die Mutationstheorie*: a work which may be said to constitute the beginning of modern genetics. Most of the changes in inherited characteristics that De Vries described—what he called mutations— are now known to be the result of changes in the grouping of the chromosomes and genes, of the sort that we have sketched.

Most of the varieties produced thus by altering the numbers and grouping of the chromosomes are not entirely permanent when reproduction occurs from two parents. The irregulari-

ties in the chromosome numbers cause irregularities in the processes of germ cell formation and fertilization, with the result that the offspring produced are not all like their parents; many of them indeed cannot live. It is only the cases in which all the chromosome pairs are doubled—so that there are four in place of each pair—that are inclined to reproduce themselves quite normally, so forming a lasting variety.

But in many plants reproduction may occur from a single parent, by cuttings, bulbs, runners, offsets and the like; these do not disturb the chromosome arrangements. Hence many of these irregularly formed varieties may be multiplied and perpetuated in this way. In cultivated plants that multiply thus vegetatively, beyond doubt such alterations in the number and grouping of the chromosomes play a large rôle in producing the great variety that is to be observed. If one goes to a Dahlia show or to an exhibition of Chrysanthemums or other cultivated flowers, he is quite overwhelmed by the extraordinary variety of forms, sizes and colors. Such an exhibition is an astonishing display of genetic variations. The differences between the differing varieties is often much greater than between many wild species found in nature. Part of these great differences are the result of crossing different stocks, thus producing new combinations of genes and of characteristics, and producing actual new characteristics, in the way described earlier. But many of the different types are the result of increasing or decreasing the numbers of certain of the chromosomes, or of all the chromosomes; of altering the grouping of the chromosomes, in the way we have just considered. A very large share of the variety seen in cultivated plants is due to such changes in grouping and combination of genes and chromosomes; resulting on the one hand from crossing different stocks; on the other from aberrations in the operation of the chromosomal mechanism.

There is strong evidence too that changes of this type play a certain rôle in producing different stocks, different varieties and perhaps different species, in plants that live in a state of nature. Plants ordinarily have one set of chromosomes from the female parent, another set, with an equal number, from the male parent. Such usual individuals may be said to have two sets of chromosomes; technically they are called diploids. As we have seen, at times individuals are produced having but one set instead of two; such are known as haploids. Other results of irregularities in distribution produce individuals with three sets, or with four sets. If we call the number of chromosomes in one full set by the letter *n,* then in ways we have indicated, there may be produced individuals carrying either *n, 2n, 3n,* or *4n* chromosomes. The production of all these different types has been observed in closely studied plants, like Datura; the different types produced having different characteristics, in flowers, fruits, stems or leaves.

In wild plants it is found that many closely related varieties or species have chromosome differences of just these kinds. Thus, in the roses the number of chromosomes in a set was seemingly originally 7. Varieties of roses are found having 14 chromosomes (two sets); 21 chromosomes (three sets); 28 chromosomes (four sets); even 35, 42 and 56 chromosomes; that is, five sets, six sets, eight sets. Again in different varieties or species of wheat the numbers go by multiples of 7; some have 14 chromosomes, others 28, others 42. The species and varieties of Chrysanthemums go by sets of 9; different ones have 18, 36, 54, 72, and 90 chromosomes. In different Oenotheras again the chromosomes go by sets of 7; in Potentillas by 8's and so on.

Similar conditions are found too in animals. In some different species of starfish the chromosomes are based on sets of 9; there are species having 18, others with 36. Some varieties

of Cyclops have 6 chromosomes, others 12. A very large number of cases of this type are known. It appears that changes in the number of sets of chromosomes present plays an important rôle in bringing about differences of type and variety, in both wild and domesticated animals and plants.

There is another method by which changes in the structure of the genetic system, changes in the grouping of the genes, produce genetic variations and add to the variety in the organic world. In any given species or variety, the genes are grouped into a certain definite number of chromosomes; in man 48, in the Jimson weed 24, in the fruit fly 8, in the horse worm 2, and so on. This grouping of the genes has an effect on inheritance, for all the genes that are grouped in a single chromosome tend to go together from parents to descendants; and similarly all the characteristics that depend on these genes that are grouped together tend to go together in inheritance. It is this tendency of characters dependent on genes in the same chromosome to go together that is called linkage.

Now, it sometimes happens that a single chromosome becomes broken into two, thus adding one to the number of groups of genes. And sometimes two chromosomes belonging to the same pair, or two belonging to different pairs, become united, so decreasing the number of groups, or chromosomes, into which the genes are united. Such changes have been observed in organisms under cultivation in the laboratories. They are now producible on a large scale by subjecting organisms to radiations. These changes produce alterations in the method of inheritance in the organisms in which they occur, for they alter the linkage, or tendency of characteristics to go together to the same individual.

And when we compare related species or varieties, we often find that they have differing groups of chromosomes, such that one could have been derived from another in one of these

ways; that is, by breaking or union of certain chromosomes. This is for example the case in different species of Drosophila and other closely related genera. Metz has given excellent figures of the chromosome groups in different species of Drosophila, some of which we have copied in Figure 8. Type A, in the common fruit fly, has four pairs of chromosomes,
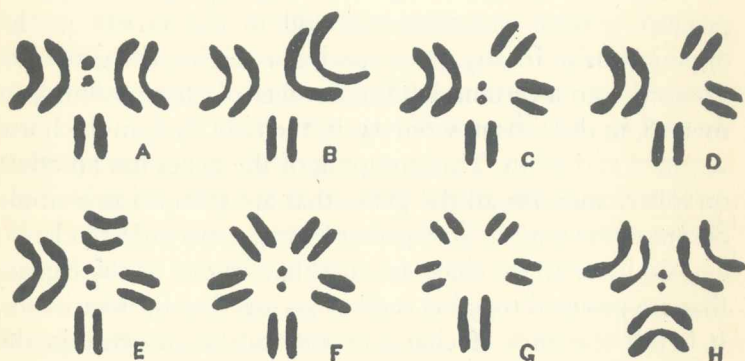
Figure 8. Diagrams showing the chromosome groups of eight different related species of flies, after Metz, 1916.

one pair straight, two pairs *V*-shaped, one pair small. Type B, in another species, is like Type A except that the small chromosomes have united with some of the larger ones, leaving but three pairs. Type C, found in a third species, is like Type A except that the chromosomes of one of the *V*-shaped pairs have broken at the point of the *V* into two straight chromosomes; so there are in this five pairs. In Type F, both the *V*-pairs of Type A have broken into two pairs of straight chromosomes, giving six pairs instead of four. The various other types result from similar rather simple changes. There are many other cases of related species having chromosome groups that thus differ by slight but definite modifications of a common type.

In other cases chromosomes of the different pairs are found to have exchanged parts; they have exchanged blocks of genes (Figure 9, *A* to *D*). Furthermore, in ways that we shall examine later, the order of the genes in a part of a chromosome sometimes becomes reversed, while in the rest of the chromosome the order remains unchanged (Figure 9, *E* and
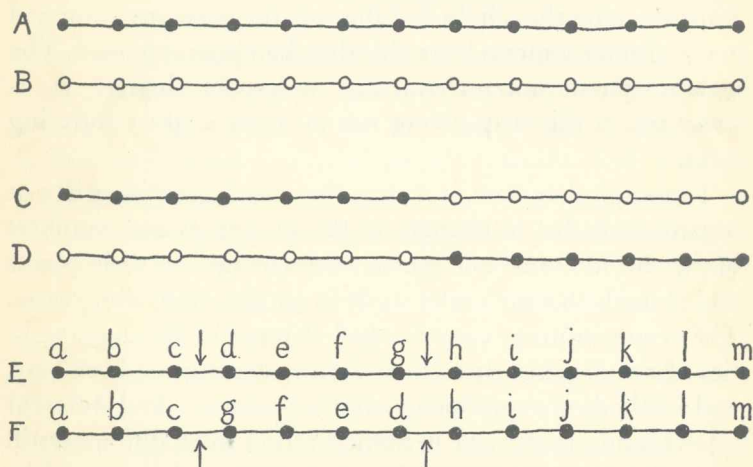
Figure 9. Diagrams of the exchange of genes between chromosomes of different pairs (*A* to *D*), and of changes in the order of the genes in a part of the chromosome (*E* and *F*). In *E* and *F* the order of the genes is given by the letters; in *F* the order of the genes between *c* and *h* is reversed as compared with *E*.

F). In this way genes that are at a distance from each other in some individuals are brought close together in other individuals. These changes in the relation of the genes, and the accompanying disturbances, result in striking changes in the method of inheritance, and also, as appears probable from recent discoveries, in the characteristics that are inherited. The genetic variations so caused appear to play a very great rôle; some of the so-called gene mutations are apparently of

this type. Further discussion of these will be reserved till we take up explicitly the subject of mutations.

But to conclude the matter with which we are now dealing, it is found that a single species may thus consist of a great number of diverse stocks, differing in the way their genes are arranged in the chromosomes, but hardly at all in other ways. It turns out on the whole that the way the genes are arranged in the chromosomes is less stable than had been supposed. The genetic system not infrequently undergoes changes in its structure, in this way giving rise to many slightly differing races.

To recapitulate then, it is clear that much genetic variety in organisms is due to changes in the groupings and numbers of the chromosomes and genes. Such changes occur in plants and animals that are under study in gardens and laboratories. They cause in many cases marked changes in the characteristics of the organisms; in their forms, structures, physiology, and methods of reproduction and inheritance. And different types found in nature or in domestication show differences in their chromosome groups that are exactly such as would be produced in these ways. We here catch nature at work differentiating genetic types, producing strains and varieties that differ in their inherent constitutions. We thus know certainly what underlies great numbers of genetic differences among organisms. We are able here to see evolution occurring; at least evolution in the sense of diversification.

But it is clear that in all these alterations in the combination and grouping of the genes, there is nothing that tends directly to bring about adaptation. In the formation of new combinations that accompanies biparental reproduction, and in the new grouping of chromosomes and genes that results from irregularities in the operation of the genetic system, unadaptive conditions are as readily produced as adaptive ones;

indeed, doubtless in the large majority of cases the results are the reverse of adaptive. Individuals produced with unadaptive groupings of genes and characters simply fail to develop, or to reproduce, leaving only such new groupings as can live and develop under the conditions. If by chance any of these have a particularly favorable constitution, these may multiply and become numerous; in this way an adaptive result may sometimes come to light. Selective elimination occurs on a grand scale in these changes; this is clearly observable in the laboratory and garden studies. Such adaptation as may result is the consequence of selective elimination and selective propagation.

Furthermore, the two types of genetic variation that we have thus far considered—recombinations of genes, and changes in the structure of the genetic system—do not involve any change in the constitution of the genes themselves. They merely rearrange the genes that already exist. In order that they shall be effective, they require the existence of many different kinds of genes. In some way diversity must arise among the genes themselves, giving rise to new types of genic materials; otherwise there could be no result from recombinations and regrouping of the genes. We are therefore put again upon a search for a third type of genetic variation, which shall change the constitution of the genes themselves.

2. GENE MUTATIONS

What have been believed to be the required actual changes in the constitution of genes, giving rise to new types of genes that make possible progressive evolution, have been observed on an extensive scale; they constitute what are known as gene mutations.[3]

In theory or concept, a gene mutation is a change in the constitution of a single gene; a rearrangement of its constit-

uent atoms so as to yield a chemically and physiologically altered material. Thus an essentially new gene is held to be produced, yielding new characteristics, structural or physiological.

Such change in the constitution of the genes would appear to constitute the primary and fundamental type of genetic variation. Without it, recombination of genes appears power-less to produce effects, for without it there are no diverse kinds of genes to recombine. If the changes in gene material are of appropriate character, they supply the remaining factor that is logically necessary for understanding evolutionary change. The transmutation, or "mutation," of the genic mate-rials, will, if it is of appropriate nature, supply new unit genes. Biparental reproduction shuffles and recombines the different genes so produced, giving organisms with diverse combina-tions of characteristics, on which selective survival and elimi-nation may act. New lasting groupings of the genes may be given by irregularities in the operation of the genetic system that we have before described. Thus with mutations of appro-priate character, there is logically a complete scheme for evolution that will yield both adaptation and progress.

But the fundamental question remains: Are the observed changes of genes indeed of such a nature as will yield progres-sive evolution? With that question in mind, we proceed to an examination of the changes in genic materials that are known as gene mutations. (We shall follow common usage in employing the term gene mutation, although on later pages its appropriateness as a designation will be called in question.)

In practice, for a change in the inherited characteristics to be identified as a gene mutation, certain conditions must be fulfilled. It must be possible to show that the change is not the result of ordinary recombinations, such as occur in Mendelian

inheritance. It must be shown farther that the change in the genetic system that gives rise to the change in characteristics is limited (at least mainly) to a single one of the genes; and that it is not simply the loss of a gene. These conditions are not by any means easy to fulfil. They can be fulfilled only in organisms in which there has been acquired a detailed knowledge of the structure and operation of the genetic system, so precise that the locations of the single genes can be determined; knowledge that would permit maps to be made, showing the relative positions of the genes. The effects of particular genes on the characteristics must be known, so that the effect of changes in these genes can be detected. Above all, the results of typical recombinations among the genes, such as occur in biparental reproduction, must be known. And finally, the results of chromosomal irregularities must have been adequately investigated. Until these things are known, it is impossible to distinguish the results of recombinations and irregularities, on the one hand, from the actual changes in single genes on the other. There are very few organisms in which these conditions have been even approximately fulfilled. The best known of these is Drosophila melanogaster, the fruit fly. As will appear in the sequel, it seems doubtful whether there has been hitherto any organism whatever of which knowledge was adequate for distinguishing gene mutations from other kinds of changes.

However, many changes have been observed that affected mainly individual genes, and that are commonly called gene mutations. Whatever their character and whatever their relation to evolution, they are in themselves of great interest and importance. A great body of detailed knowledge on these has accumulated. We shall first deal with the main features of this established knowledge. Next we shall make an explicit examination of the question that is of underlying interest for

evolution: whether the observed changes in the genes do indeed provide the fundamental materials for progressive evolution.

The changes known as gene mutations have been found to occur in genes that affect all parts and functions of the organism. Indeed, in the organism in which the course of heredity is best known—the fruit fly—practically all knowledge of heredity is based on the mutations that have occurred. The ordinary "wild" individuals are extremely uniform; mating two of these, the progeny are uniform and there is little opportunity for the study of the rules of inheritance. But when a gene becomes mutated, and the individual carrying it is mated with another in which that gene is not mutated, the descendants display all the rules and proportions of Mendelian heredity. In this way the course of heredity for hundreds of structural and physiological characteristics has been worked out; in each case there has been a mutation in a gene affecting the characteristic.

The same single gene becomes mutated in different individuals in different ways, so as to give different characteristics in each case. So, in Drosophila there is a gene located near one end of the X-chromosome, which cooperates with other genes in producing the color of the eye. If this particular gene and the others that work with it are in their usual or "normal" condition, the color of the eye is red. If this gene is mutated in a certain way (the other genes remaining unchanged), the eye color changes to white. Other mutations in this same gene give other eye colors, and in this way, by different mutations of this single gene a whole series of eye colors have been produced, some eleven or twelve in all. These eye colors, resulting from different mutations of this one gene, have been given the following names, beginning with those

showing the deepest colors: red, coral, blood, cherry, eosin, apricot, ivory, tinged, buff, écru, white, and ultra-white.

Other genes in Drosophila and in other organisms, are known thus to have been changed by mutation in many different ways; many series of modifications of a single gene, like that just listed, are known.

The change induced by the mutation of a gene may be great, or it may be very small. The eye of the fruit fly is changed by a certain mutation from red to white; a great change. By another mutation it is changed in a barely detectable way, from red to "coral" or "blood." Based largely on the so-called mutations of De Vries—which were in fact mainly the result of gross chromosomal irregularities—there has been a prevalent tradition that mutations are sudden extensive changes, "saltations," like the change from red eye to white eye. It was natural that the first mutations observed should be these very conspicuous changes: sudden loss of wings or of eyes; or other marked changes in structure. Such conspicuous mutations are indeed not infrequent.

But as studies became more exact and detailed, it was found that many mutations produce extremely minute changes, not to be detected without great care. For example, there are known in the fruit fly a number of gene mutations whose only discoverable effect is to cause a very faint lightening of the color of the eye in case the individual containing them has eosin-colored eyes. If the eyes are not eosin-colored, these mutations have no discoverable effect. The number of gene mutations having such very slight effects is much greater than that of those having marked effects; most mutations produce almost imperceptible changes.

The majority of mutations are recessive in heredity. That is, if in one of the pairs of genes, one of the two has been mutated, the other not, the mutated gene produces no mani-

fest effect on the individual; the latter remains quite un-
changed. Thus one or many gene mutations may have
occurred in the chain of genes of a given individual, but so
long as only one gene of any pair is affected, there may
be no manifest effect. Only when both members of a pair of
genes are mutated does the recessive mutation produce its
bodily effect. A small proportion of the mutations that occur
are dominant; in this case even when but one member of a
pair of genes has mutated, the individual is changed by the
mutation.

As to their absolute frequency, in ordinary observation the
changes known as gene mutations seem to occur very rarely.
Yet when large numbers of individuals are examined for
long periods, and the eye is carefully trained for the detection
of mutations, the number occurring in even a short period of
years is very considerable. In the fruit fly, during twenty-five
years of observation, such changes have been seen to occur in
several hundred different genes. In relation to the course of
evolution, twenty-five years is an infinitesimally small period,
so that relative to the course of evolution, gene mutations are
numerous and of frequent occurrence. If they fulfill other
requirements, their numbers are sufficient to account for
evolution.

As to the occurrence of gene mutations, a mutation occur-
ring at a given time appears to affect only a single one of the
genes in the chain of genes. Further, a mutation occurs in
only one of the two like genes that are present in any cell.
The genes are in pairs, the two members of a pair having
similar or identical constitutions and effects. But the occur-
rence of a mutation affects but one of these, leaving the other
unchanged. It can even be shown that when a single gene has
split in two, in preparation for cell division, one of the two
halves may become mutated while the other does not. All

these relations show that whatever is causing the mutation is precisely localized and extremely restricted in its action.

Since most mutations are recessive in their effects, and since only one gene of the pair of genes present is mutated, as a rule the mutation produces no manifest effect on the individual in which it occurs. The fact of its occurrence cannot be detected in that individual. It is only when by the processes of mating in ordinary reproduction, two of the recessive mutations get together in the same pair, in some of the descendants of this individual, that the mutation appears to view.

Thus, when the recessive mutation becomes manifest, it has actually occurred, as a rule, at least two generations earlier, in one of the ancestors of this individual. This relation introduces much difficulty into the study of the time and place at which mutations occur. Many mutations do not come to light for several or many generations; indeed there must exist in any organism many mutations that have never come to light. In the course of generations a large number of recessive mutations may have collected in the chromosomes, none of them producing any effect, because each is accompanied by a normal or unmutated gene of the same pair. If now inbreeding—the mating of close relatives—occurs, a number of these mutated genes may be brought together into the same pairs, in one individual; thereupon the effect of all these mutations suddenly become manifested in that individual. Since most mutations are harmful in their effects, this individual will be weak and defective, perhaps in several different ways. This is the reason why inbreeding is harmful; if there were no gene mutations, apparently there would be no disadvantage in the mating of close relatives. One of the chief results of biparental reproduction—what might be called one of its functions—is that it largely nullifies the effect of harmful mutations.

In view of the fact that mutations may remain long hidden, it is fortunate for purposes of study of the occurrence of mutations that in many organisms a certain number of genes in one of the chromosomes, known as the X-chromosome, are in one of the two sexes not paired, but single. In most organisms that have been extensively studied from this point of view, it is the male that has a certain number of genes that are single, in the X-chromosome. If any of these unpaired genes become mutated, in the fertilized egg or in the cells from which the ovum is derived, the effect of the mutation appears at once, in case the individual developed from that egg is a male. Thus the time and place of the occurrence of gene mutations has been studied most extensively in the genes of this X-chromosome. In recent years, however, an elaborate and adequate technique has been devised for bringing to light almost at once the effect of mutations in the genes of any of the chromosomes. In this way much has been learned as to the time and place of the occurrence of mutations, and as to the agents causing them.

It has been discovered that a gene mutation may occur in any cell of the body, at any period in the life of the organism. The mutation occurs in but one single cell. If this is the single cell of the fertilized egg, then all the cells of the body developed from that egg will carry the mutated gene. If the cell in which the mutation occurs is one of the two cells into which the fertilized egg divides, then only half the body will carry that mutation. If the mutation occurs in a cell at a still later stage of development, only a small part of the body will show the mutation. Many cases of such kinds have been thoroughly studied, cases in which one part of the body shows the mutation, the rest not. The general upshot of all these studies is to show, as remarked before, that gene mutations may occur in any cell of the body, at any period in life.

From this it follows that many gene mutations occur that have no manifested effect whatever; they do not change the characteristics of the individual in which they occur, nor those of his descendants. For example, consider a mutation in a gene that affects the color of the eye. Eyes derived from the cell in which this mutation occurs will be changed in color. But many of the cells of the body do not produce eyes; instead they produce legs, wings, parts of the body, or the like. Mutation of an eye-color gene in such cells will thus have no effect on eye color, no visible effect on any characteristic. It can probably be said with truth that most eye-color gene mutations have no effect on eye color, for most of them occur in cells that do not produce eyes. Doubtless there occur great numbers of such mutations that yield no visible effect; any individual may carry many such mutated genes. All such mutations, present in body cells only, are totally lost at the death of the individual carrying them.

When a mutation has occurred, is the change a permanent one? Or may the genetic system later return to its original condition?

The great majority of mutations appear to be permanent changes. They are inherited by the progeny of the mutated individuals, and such inheritance continues for an indefinite number of generations.

But in recent years a considerable number of cases have been discovered in which the change is not permanent. Having mutated in a certain way, producing its usual effects on the characteristics, the genetic system later changes back to normal, so that the original characteristics are restored. Sometimes such a reversion to normal is produced by subjection of the mutated genes to radiation. In other cases the mutated genes show a marked tendency to revert to the original condition; reversion occurs in a considerable proportion of them,

without the action of radiation. A considerable number of mutations that behave in this way have been discovered. This is a matter of much interest in relation to the nature of mutations; some examples will therefore be mentioned.

Demerec[4] found in a species of Drosophila that a mutation affecting a certain gene located at a particular point in the X-chromosome caused the body to take on a reddish tinge, instead of the normal gray. Another mutation of this same gene caused the body to become yellowish in color. When these two differently mutated genes were brought together in the same cells, it was found that in about one-fifth of all the cases in which this was done, the genes that had mutated to reddish were transmuted back to the original condition, so that they produced again the original gray color. Thus the mutation that produced the reddish color was not a permanent change. In some way the presence with it, in the same cell, of the gene that had mutated to yellow caused in some cases the reversal of the change to reddish.

The proportion of the cases in which the reddish gene changes back to normal was found to differ in different families. By selection from the different families and further breeding, Demerec obtained families in which the reddish mutation never returns to normal; others in which the reversion occurs very frequently.

Other reversible mutations were found in the same animal. A mutation of a certain gene ("miniature") in the X-chromosome caused the wings to be small.[5] In later generations this mutated gene frequently reverts to normal. By selection from different families it was possible to obtain some stocks in which the return of the mutated gene to normal takes place very rarely, the mutation remaining permanent in most cases. In such a group there were but five reversions in 11,609 cases. In other stocks the reversion is very frequent, occurring in up

to 50 per cent of the cases. The reversion may occur in either the germ cells, or in certain of the body cells. When the reversion occurs in the germ cells, the offspring of the mutant (small-winged) individuals are of the normal large-winged type. When the reversion occurs in the body cells, those parts of the wings derived from these reverted cells are of the normal large type, while the remaining parts of the wings are of the miniature type. Thus mosaic individuals are produced, portions of the wings large, other parts small, in the same individual. In certain families reversion from mutated to normal occurred frequently in the body cells, yielding mosaic individuals; but never in the germ cells.

Extreme cases of frequent, reversible, and inconstant mutations are known in plants. Typical examples of these are seen in variegated maize or Indian corn.[6] In the variegated varieties, parts of the same ear of corn show seeds of different colors: red, yellow, white. Even single seeds on the ear may be variegated; splashed or streaked with two or three colors in small areas. The variegated condition is known to be due to a single gene that differs from one of the genes in the uniform varieties.

When the variegated plants are bred together they give a number of different sets of offspring, with different types of coloration. Some of the offspring have the seeds mainly of one color; some mainly of another color; some much variegated, some all of one color. The diversities of color tend to be inherited, showing that the differences in color are due to differences in the genes. If from a variegated ear there be selected two seeds, one lighter in color, the other darker, the former gives lighter offspring, the latter darker offspring—each still with much variation. From a single seed of a certain single color a plant may be produced having diverse colors in its

different seeds, and these colors are (partly) heritable in later generations.

Thus in these plants the genes frequently mutate so as to yield at one time one color, at others another. The mutations occur in many different ways, yielding many different colors and patterns of variegation.

These facts as to frequent, reversible and inconstant mutations raise questions as to the essential nature of mutations. What is it that is really occurring here? To this question we return.

What are the agents that produce mutation? For many years after mutations were found to occur, nothing was known as to the agents that cause them. A few genes mutated, as a few of the atoms of a radiant metal disintegrate; in both cases no outside agent appeared to be at work. There seemed to be no relation between the environment and the production of genetic variations.

But of late this situation has changed. The connection between environmental action and genetic variation has been made. Certain outer agents are found to play a rôle in producing gene mutations. In 1927 H. J. Muller[7] discovered that by subjecting developing organisms to a powerful dose of X-rays, the genetic system was altered in many ways. The X-rays injure the animals; they kill a large proportion of them; they leave many of the rest sterile. Still other survivors are found to have suffered changes that leave their descendants with hereditary abnormalities, weaknesses and other peculiarities; and these are found to be the result of changes in the genetic system caused by the radiations, changes that are known as gene mutations. The radiations have caused the chromosomes to break, caused pieces from different chromosomes to stick together; destroyed some of the genes; produced irregularities of many kinds. And amid all this wreckage, they have pro-

duced a lot of gene mutations, to which are due the inherited abnormalities, weaknesses and other aberrations. The number of visible mutations produced under the action of radiation is about seventy-five times as great as occurs without radiation.

This effect of radiations in producing mutations was confirmed by others and extended to other organisms. Radiations from radium have the same sort of effect as X-rays; they greatly increase the number of gene mutations. Gene mutations have been produced by radiations in maize, barley, tobacco, cotton, various animals, and many other organisms.

By radiation the same types of mutations are produced as occur spontaneously. In many cases the mutations due to the radiations are identical with others that had occurred without radiation. Others induced by radiation differ from any before observed. The same gene may be caused by radiation to produce repeatedly in different individuals the same mutation. Since all kinds of mutations are induced by radiation, it is clear that there is here no relation between the kind of mutation produced and the kind of environmental agent that induces them. The mutations induced by radiation are inherited in later generations, as are those that occur spontaneously.

The number of mutations produced in a given number of individuals is found to increase in proportion to the energy of the radiation; that is, to its intensity and duration, up to an energy that causes injury so great that the radiated germ cells do not develop.

The question arises as to whether the "spontaneous" mutations are not partly or wholly the result of radiations occurring under natural conditions. It was found that fruit flies kept in a mining shaft in which natural radiation from the minerals present was abundant gave a larger proportion of mutations than those kept under usual conditions, indicating

that the natural radiations are indeed effective in producing mutations. But a very careful study by Muller and Mott-Smith, based on the fact that the proportional number of mutations varies as the intensity and duration of the radiation, showed that the number of mutations that occur under natural conditions is much greater than can be accounted for by the frequency of natural radiations. It is clear therefore that some other agent is at work.

And another agent has been identified. Muller found that the number of mutations is slightly increased when organisms are kept at a high temperature—29 degrees in place of 20 degrees. Then Goldschmidt, and after him Jollos,[8] employed heat that was destructive; up to 37 degrees. Again, as in the case of radiation, most of the organisms are killed. Again the survivors show injuries and weaknesses of various kinds. And again the progeny of survivors show inherited abnormalities and peculiarities which are found to be due to gene mutations. The same types of mutations are produced by heat as occur spontaneously and under radiation; they are inherited in the same manner.

Certain other matters of great interest have been reported by Goldschmidt and Jollos in connection with the mutations induced by heat. In some of their experimental cultures very great numbers of mutations were produced. It was further reported by these authors that the heat produces changes in the bodies of the individuals subjected to the heat, and that in later generations similar inherited changes appear as a result of gene mutations. However, these later mutations did not as a rule appear in the descendants of the particular individuals that had been directly changed by the heat.

In the continuation of this work by Jollos, a further relation, of the very greatest interest, is reported. Subjection of one generation to a high temperature causes a slight inherited

effect, due to a slight mutation of a particular gene. Subjection of those changed descendants to the high temperature for another generation is reported to increase the effect and alter the gene still more. By continued subjection to high temperatures for many generations a series of gradations is thus produced, each one hereditary at ordinary temperatures, but passing from slight changes in earlier generations to very great ones in later generations. Thus, in one series of experiments, a certain gene was so altered, in the first generation subjected to heat, as to cause a slight darkening of the body color. Subjection to heat in later generations increased the effect on this gene until finally the body was black. In another series, the red color of the eye was, by subjection of successive generations to heat, caused to become lighter and lighter until it was yellowish, and until finally entirely white eyes were produced. The mutations to which these changes in eye color were due were all in a certain gene, that is perhaps the best known gene in the fruit fly. Thus what could be called directed mutations were induced; series of changes in a certain direction for successive generations; what might be called experimental orthogenesis.

Furthermore, according to Jollos, the nature of the mutations was dependent to some extent on the nature of the conditions in which the organisms were subjected to the heat. Those in moist conditions, for example, gave a different set of mutations from those in dry conditions.

These rather revolutionary conclusions are, however, not confirmed by the extremely extensive and long-continued work of Plough and Ives.[9] These authors confirm the fact that high temperatures increase the frequency of mutations. But they do not verify the other conclusions of Jollos; and particularly they find no indications of the directed muta-

tions, or orthogenetic series reported by Jollos. As matters stand, the more radical conclusions of Jollos cannot be considered established.

The discovery of agents producing mutation brings us squarely to the question in which these lectures are primarily interested. Both the known agents are powerfully injurious. Both kill a large proportion of the individuals exposed to them; both leave many of the others abnormal or imperfect. Both damage the genetic system, producing changes that make it impossible for the individuals to develop. And both produce inherited changes that are manifested mainly as abnormalities, weaknesses and imperfections.

Are these the type of changes on which evolution is based? Amid the carnage engendered by blighting radiation and unbearable heat, do we find produced the changes that mean progressive evolution? The gene mutations produced by radiation and by heat are of the same types as occur spontaneously. It is commonly assumed and urged that the mutations observed in experimental genetics are the raw material of evolutionary change; are visible steps in evolution. Is this to be considered established? This question we take up in our next chapter.

NOTES ON CHAPTER II

[1] Page 27. Changes and irregularities in the genetic system in relation to the differentiation of varieties, species, etc., have been very fully described and figured in C. C. Hurst's *The Mechanism of Creative Evolution* (New York and Cambridge, 1932). See also T. H. Morgan, *The Theory of the Gene* (New Haven, 1928) and *The Scientific Basis of Evolution* (New York, 1932).

[2] Page 30. The work on Datura is by Blakeslee and his associates. See A. F. Blakeslee and J. Belling, "Chromosomal Mutations in the Jimson Weed, Datura stramonium," *Journal of Heredity,* Vol. 15, 1928, pp. 195-206; and the references there given.

[3] Page 41. For general accounts of gene mutations, see the references given in Note 1, above.

[4] Page 50. See M. Demerec, "Reddish—A Frequently 'Mutating' Character in Drosophila virilis," *Proceedings of the National Academy of Sciences,* 1926, Vol. 12, pp. 11-16.

[5] Page 50. See M. Demerec, "Miniature-Alpha—A Second Frequently Mutating Character in Drosophila virilis," *Proceedings of the National Academy of Sciences,* 1926, Vol. 12, pp. 687-90.

[6] Page 51. For an account of variegation in Maize, see W. H. Eyster, "A Genetic Analysis of Variegation," *Genetics,* 1924, Vol. 9, pp. 372-404.

[7] Page 52. See H. J. Muller, "Artificial Transmutation of the Gene," *Science,* 1927, Vol. 66, pp. 84-7, and "Types of Visible Variations Induced by X-Rays in Drosophila," *Journal of Genetics,* 1930, Vol. 22, pp. 299-334.

[8] Page 54. See V. Jollos, "Studien zum Evolutionsproblem. I. Ueber die experimentelle Hervorrufung und Steigerung von Mutationen bei Drosophila melanogaster," *Biologisches Zentralblatt,* 1930, Bd. 50, pp. 541-54.

[9] Page 55. See H. H. Plough and P. T. Ives, "Heat-Induced Mutations in Drosophila," *Proceedings of the National Academy of Sciences,* 1934, Vol. 20, pp. 268-73.

CHAPTER III

ARE THE GENE MUTATIONS OBSERVED IN EXPERIMENTAL GENETICS THE BASIC MATERIALS OF PROGRESSIVE EVOLUTION?

WE TAKE up in the present chapter the question proposed at the end of the preceding. Do the gene mutations observed by the students of genetics constitute the types of change of genic materials that are required for evolution; and particularly for progressive evolution? Are they steps in evolutionary progress?

Here we frequently meet an argument from definition. Evolution, it is urged, requires, and is finally based on, changes in the constitution of the genic materials. And any change in the constitution of the genic materials would be by definition a gene mutation. Hence it argued that evolution certainly rests on gene mutations. But such argumentation has no relation to observed facts; it was equally valid before any mutations were seen. The question of interest is: Has genetic science actually before it steps in evolution? Do the *observed* gene mutations constitute the type of change that has resulted in progressive evolution? It is to this question that we shall address ourselves. Our discussion relates only to observed types of change; not to conceivable but unseen types.

What may be a definite answer to these questions is now emerging before us. On it there are several lines of evidence. First is the fact, already set forth, that the agents known to produce gene mutations are definitely destructive agents. They injure the organism in all its parts and functions, including its genetic parts and functions, including its germ plasm. By an astounding paradox, we are asked to believe that

they also produce the changes on which evolutionary progress is based.

But more probative than this are two other lines of evidence. One lies in the observed effects of gene mutations on organisms. The other lies in an experimental analysis of the nature and causes of mutational change; in the recent uncovering of some of the hidden processes occurring at mutation, showing just what is happening. We shall look into both of these.

From the first it has been observed that when carefully examined most mutations are discovered to be defects. The organism is the worse for their occurrence. They are not of such a nature as to yield adaptations, but the reverse. The changes that they represent are not progress, but the opposite.

These relations are shown in two ways, each of great importance. First, the specific effect of the mutation on some particular part or function of the organism is, in most cases, to bring about a distinct loss, or an abnormality. Certain mutations cause the body to be distorted, or imperfect in parts, or to lose certain typical structures. Others make the limbs abnormal or small, or result in their total loss. By other mutations the eyes are made imperfect, or very small, or are totally lost. All these are well known and typical results of gene mutation: great numbers of such cases could be cited. In general it is true that a part that has been altered by a mutation is not so well adjusted to the rest of the organism, or to the surrounding conditions, as it was before the mutation occurred. Whether there are any exceptions to this we consider later. But certainly the general impression from a group of mutated organisms is one of defectiveness and abnormality.

Second, and perhaps still more significant for the nature of mutations, is the fact that, in addition to their specific effects on particular parts of the body, mutations usually have a

general effect on the organisms as a whole, and this effect is injurious. They distinctly weaken the constitution of the individuals in which they exist. The result is that the mutated individuals lack resistance to severe conditions, and have a shorter life and a higher mortality rate than those not mutated. It is a common observation that individuals showing the mutated characteristics are difficult to keep alive; specially favorable conditions must be supplied or they perish. Indeed, a large proportion of the mutations that occur cause a complete failure of development, unless the mutated gene is accompanied by an unmutated gene of the same type. Such are known as lethal mutations. One is surprised, when he comes to study the occurrence of mutations, to discover that the commonest types are these lethal mutations; a large proportion of our knowledge is based on these changes that cause death. Often when one gene of a pair is thus affected by a lethal mutation, while the other is normal, the individuals are weak and abnormal in structure and function. If both genes of the pair are affected by the mutation, the individual "dies before it is born."

There occur gene mutations whose effects, both special and general, are so slight that from examination of the individuals affected by them, it is difficult to determine whether they are harmful or not, although as a rule the general impression they give is one of slight abnormality or loss. But here the correct interpretation is aided by certain extremely ingenious experiments made possible by the action of radiation on chromosomes and genes. The results of these experiments are illuminating; we must examine them.[1]

As will be recalled, normally there are in the cells of any individual two genes of each type, forming a pair. That is, there are present two "doses" of the material represented by the gene. By the use of radiations it is possible to obtain indi-

viduals having but one dose of any particular type of gene material, and others having three doses (Figure 10). An individual carrying a pair of mutated genes, one in each of its chromosomes, is subjected to radiation. By this, a small
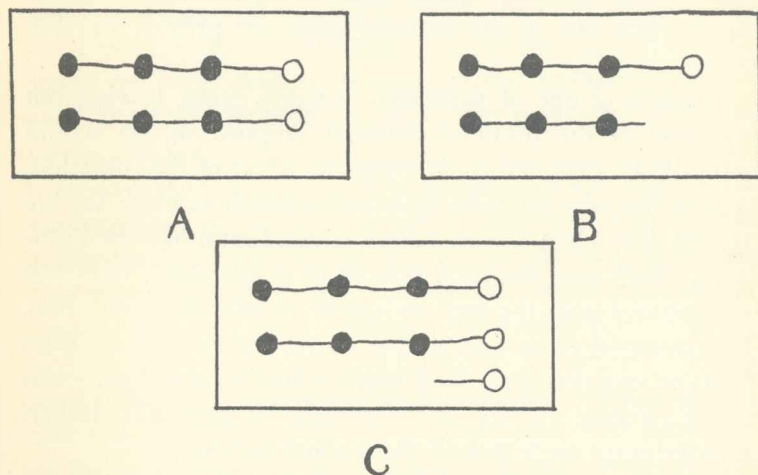
Figure 10. Diagrams of a small portion of the chain of genes, showing how it is possible to compare the effects of one, two or three mutated genes. *A.* Normal condition, two mutated genes (at right). *B.* One mutated gene removed. *C.* Three mutated genes present.

portion of one of the chromosomes, carrying the mutated gene, can be removed, leaving the organism with but one dose. Or by radiation a small piece of the chromosome containing the mutated gene is broken off, and later by proper breeding introduced into an individual that already has the usual two doses; so that now it contains three. Thus it becomes possible to compare the effects of one, two, or three doses of a certain mutated gene.

An example will illustrate the nature of the relations discovered in such comparisons. The eye of the fruit fly is

normally a deep red in color. When a mutation has occurred
in a certain gene that is near the left end of the X-chromo-
some, this causes the eye to change to a lighter color, known
as eosin. The effect of the mutation is thus to lighten the
color of the eye. This lighter color, being recessive, is mani-
fested when two of the mutated genes are present together
(Figure 10, *A*).

Removal of one of these two mutated genes, leaving but
one dose of the mutated material (Figure 10, *B*) would
naturally be expected to decrease the effect of the mutation,
causing the color not to be lightened so much as when two are
present. But when this was done, it was found that the light-
ening effect was increased instead of diminished; with but
one mutated gene the eyes are lighter in color than with two.
Two mutated genes give a color nearer the normal red than
does one mutated gene. And when a third mutated gene was
added (Figure 10, *C*), the eye color became still darker,
approaching closely that of the normal red eye.

It thus becomes clear that the effect of the mutation has
been to reduce the normal action of the gene, so that more
doses of the mutated gene are required to produce the deep
color than are required of the unmutated gene. The mutated
gene has the same kind of action as the normal gene, so far
as it goes, but its action is imperfect, incomplete. For it is
found that the presence of only one dose of the mutated gene
leaves the individual very different from the normal unmu-
tated individual. If two are present, the result approaches
more nearly that of the normal gene, while if three of the
mutated genes are present, the individual becomes almost
like one in which there is no mutation.

It will be recalled that this eye gene is one that has been
mutated in many different ways, yielding many different
colors. One of the other mutations, giving a still lighter color,

known as *apricot,* has been tested in the same way, with the same results. One dose gives a very light color; two give a darker color; three give nearly the normal color. This mutation too is a reduction in the action of the normal gene. The entire series of mutations giving different grades of color are different degrees of reduction of action of the normal gene. Whether it is conceivable and possible that in some of the mutations one part or feature of the normal activity is reduced, while in another, another part is reduced, I will not attempt to say.

In some cases it is not at first obvious that the effect of the mutation is a reduction in action as compared with the normal gene, but experiments of the type just described show that this is indeed the case. The body of Drosophila carries a large number of simple bristles. By mutation of a certain gene, some of the bristles are caused to be forked instead of simple. But this forked condition is most marked in individuals that have but one of the mutated genes (the other gene of the pair being completely absent). If two of the mutated genes are present, the forking is still perceptible, but is not quite so marked. But if three mutated genes are present, the bristles are much less distinctly forked; they are more like normal simple ones. It seems that the forking is due to the fact that certain normal developmental processes are in the mutated individuals not fully carried out; the effect of the mutation is to reduce the normal action. The greater the number of mutated genes present, the more nearly the normal processes are carried to completion.

Tests of this kind, so far as carried out, indicate that most mutant genes are of this type. The mutational change consists in a reduction in the activity of the normal gene. So far as this is true, mutation is process that is not constructive, but destructive; not progressive, but reductional.

It agrees well with this fact that most mutations are recessive, as compared with the effect of the normal gene; so that when the mutated gene and the normal gene are present together, the effect on the characteristics of the organism is that of the normal gene alone. There do occur, however, mutations that are dominant; they produce a positive effect different from that of the unchanged gene; an effect that is manifested even when the unchanged gene is present. But such dominant mutations not only as a rule are manifested as marked imperfections or abnormalities, but also they are seriously injurious to the constitution of the individual, so that if two doses of the dominant mutation are present usually the individual dies or fails to develop at all.

On the whole it is certain that the overwhelming majority of gene mutations are not adaptive, not progressive, but the reverse.

But are there no gene mutations that are beneficial to the organisms in which they occur? Are there none that leave the organism more vigorous than before their occurrence? Are there none that are progressive in character?

It is possible to collect from the literature of genetics a few cases in which gene mutations are held to be possibly or probably beneficial. In the cotton plant there are two varieties, one having forked or deeply cleft leaves; the other, leaves that are nearly entire, being but slightly lobed. Horlacher and Killough[2] observed that under radiation a mutation from forked leaves to the lobed leaves was produced. As the lobed leaves have more leaf surface, Horlacher and Killough hold that this mutation may have been beneficial.

Again, in the production of mutations by high temperatures, one of the mutations is from the red eye color to white eye; a common mutation also under radiation, and occurring too under natural conditions. It is a typical loss mutation,

resulting in weakening of the constitution, so that under ordinary conditions the white-eyed flies show higher mortality than the normal red-eyed. But Jollos[3] finds that the white-eyed flies have a greater resistance to high temperatures than the normal individuals with unmutated red eyes. This is, so far as I know, the only case of a mutation held to increase the resistance of the organism to bad conditions.

Other cases of mutations held to be advantageous could doubtless be culled from the literature. But they are rare, and the evidence they present is not impressive. From them a convincing case cannot be made out for beneficial and adaptive mutations.

But with relation to progressiveness, we find it commonly stated as an established fact that not all mutations are reductional, although most of them are. Some of them, it is asserted, are progressive.[4] But when we look at the examples of such progressive mutations, what do we find? The examples given are what are known as reverse or return mutations. A normal gene has mutated in the usual reductional manner. In some cases it later returns to the normal condition, and this is called a reverse mutation. As before described, a considerable number of such reverse mutations have been observed, some occurring spontaneously, others under radiation. Now, if the original mutation was reductional, then, it is urged, the reverse mutation—the return to normal—must be progressive. This is logical, but what is its relation to the question in which we are here interested? A reverse mutation, it is clear, gives no example of a change producing progressive evolution. It restores the original condition, but does not carry things farther. If mutations are essentially reductions, injuries or abnormalities, the return mutation is merely a cure of the original injury. If there are mutations that yield progressive evolution, they must be of a different character from this.

Pause now for a moment to look back at the history of the idea that gene mutations furnish the steps in progressive evolution. You will recall that for many years, and until comparatively recently, nothing was known as to the agent causing mutations; they appeared to occur "spontaneously," or from inner causes.

It was observed that most of them—at least most pronounced mutations—are injurious in effect. But, it was set forth, this is doubtless to be expected. Assuming that these are undirected changes, it is natural that marked chance alterations occurring in a complicated system such as an organism should as a rule be harmful. But among the great number occurring, there will be some few, it was urged, that turn out to be advantageous; it is these that furnish the steps in progressive evolution. Moreover, it could be pointed out that there are great numbers of slight mutations that are not obviously disadvantageous or reductional. This line of argument was plausible, was reasonable; it was generally accepted.

Something of a blow was given to this conception of the matter when it was found that the agents that produce gene mutations are powerfully injurious; that they kill most of the individuals subjected to them, and that they damage the survivors in all sorts of ways. Particularly remarkable did it appear that they damage the genetic system, breaking up the chromosomes, destroying some of the genes and so producing deficiencies; injuring others so that they will not support development, and others so that they produce inherited losses and abnormalities. It seemed a paradox that among *these* things we should find the progressive changes that give evolutionary advance. But some paradoxical things are true, and the theory stood up under the burden thus laid upon it.

A distinct addition to this burden came when it was shown that the slight mutations, not obviously disadvantageous, are in fact reductional in character; that the mutated gene is in fact a weakened gene, giving effects in the same direction as the normal one, but less efficient.

Heavy further blows to the theory that the observed types of gene mutation furnish the steps for progressive evolution come with the recent discoveries which permit an analysis of the nature of the changes that are occurring in what are called mutations. These discoveries lie in two fields. On the one hand, subjection of organisms to radiations, with the production of typical mutations, has permitted a detailed experimental analysis of what is really happening. On the other hand, discoveries have lately been made that give an unexpected insight into the structure of the chromosomes, making it possible to determine what kinds of changes have occurred after a mutation. We look first at the analysis permitted by the results of radiation.

Under radiation it is found that the number of gene mutations produced is proportional to the energy of the radiation to which the organisms are subjected; also to the number of free electrons produced by this amount of radiation. In view of this fact, it was judged probable that a mutation was the result of the striking of a gene by a free electron. It was held that when an electron strikes a gene, this causes a transformation of its molecular structure, in consequence of which it acquires new properties and so gives rise to changed characteristics.

But besides gene mutations, radiation produces other effects on the genetic system. It breaks the chromosomes. It destroys certain parts of them. It sets free small pieces of them. It causes irregularities of many kinds in the structure and operation of the genetic system.

And now it has been discovered that there is close relation between these gross destructive changes and the production of gene mutations. Two of the chief investigators in this field, H. J. Muller and L. J. Stadler, recently published simultaneously two papers calling attention to these remarkable and significant relations.[5] The paper of Stadler develops in detail the fact that gross injuries and gene mutations grade one into the other, and that there is no sound basis for making a distinction in kind between them. The paper of Muller goes into a fuller analysis of the matter; I shall follow it in the main.

As to the relation between chromosome injuries and gene mutations, there are a number of facts that are of great significance. The number of breaks and injuries produced by radiation is proportional to the energy of the radiation. So also, is the number of gene mutations. Indeed, mutations occur with about the same frequency as the breaks and injuries. And what is still more significant, gene mutations commonly occur in the same chromosomes that are broken or otherwise injured by the radiation. When a chromosome is broken under the action of the rays, commonly a mutation occurs in a gene very close to the point of breakage.

Both the mutations and the chromosome breaks are extremely rare, absolutely considered. They occur in only one chromosome out of hundreds exposed to the rays. Their frequent occurrence together therefore cannot be accidental. They are in some way causally connected. Whatever causes a break in a chromosome commonly at the same time causes a mutation. And it causes a mutation at or close to the broken end of the chromosome.

Now, there is strong evidence as to how chromosome breaks are caused. And since gene mutations are similarly caused, we need to examine this evidence. It arises from cer-

tain curious relations in the occurrence of chromosome breaks
and injuries.

While under radiation, the chromosomes are long threads
bearing the genes at intervals. In well studied organisms, the
order in which the different genes occur on the thread is well
known, so that maps of the genes have been constructed.

When through the action of radiation there occurs in a
chromosome a break at a certain point, it is an extraordinary
fact that there is usually or always in the same cell another
chromosome break, at some distance from the first mentioned
one. This other break may occur at another point in the same
long chromosome, or in another chromosome of the cell. This
is another strange fact, worthy of full consideration.

Chromosome breaks occur in very few of the cells, abso-
lutely considered. The fact that there usually occur two breaks
in one cell cannot be due to chance. Whatever causes one
break causes also another. The chance of a single electron's so
striking the chromosome as to cause two breaks some distance
apart is so minute that it can be excluded.

And now another strange fact. After the two breaks have
occurred, the pieces separated by the breaks usually unite by
their broken ends, often in a different order from before. In
the case of a single chromosome that has broken in two places,
thus yielding three pieces, the three pieces often reunite in
such a way that the middle piece is reversed in position
(Figure 11, *B*). That is, the order of its genes is the opposite
of that which prevailed before the break and reunion; this
can be determined by breeding tests. A great number of such
inversions of the middle part of the chromosome are known
—the order of the genes reversed in the middle piece, though
not in the two end pieces. This is an extraordinary phenom-
enon; it cries out for explanation.

In other cases the middle piece drops out entirely and is lost. The two end pieces then reunite at their broken ends. Thus a short chromosome is produced, lacking all the genes of the middle region, but still carrying those near the two ends (Figure 11, *C*). These are known as deletions—the mid-

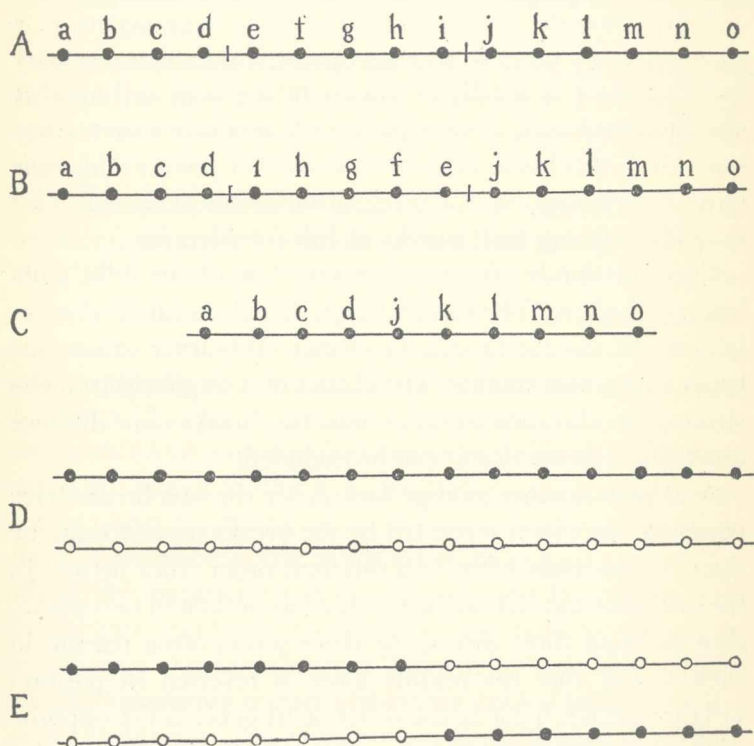*Figure 11.* Diagram of changes in the order or arrangement of the genes in consequence of breakage of chromosomes. *A.* Part of a chromosome with genes *a* to *o* in the normal order. *B.* Inversion: the genes between *d* and *j* are reversed in their order. *C.* Deletion: the genes *e* to *i* have dropped out. *D.* Genes of two chromosomes. *E.* Translocation: the two chromosomes of *D* have exchanged certain portions.

dle part being deleted. A large number of such deletions are known. The process by which they are produced demands explanation.

A third strange result is often produced when the chromosomes are broken by radiation. If the usual two breaks are in two different chromosomes of the same cell, then frequently these two chromosomes exchange parts. The broken end of one piece from one chromosome unites with the broken end of one of the pieces from the other. The two other pieces of the two chromosomes similarly unite. Thus we get two newly combined chromosomes, each made up of two halves that formerly belonged to two separate chromosomes (Figure 11, D and E). Such exchanges, or translocations as they are called, are known in many cases. Their formation calls loudly for explanation.

Thus several questions demand answer. First, how does it happen that in a cell, two chromosome breaks occur instead of one? Second, how do the broken ends find each other and unite, often resulting in a new combination? And third, how does it happen that in such reunited chromosomes the order of the genes in the middle piece is commonly reversed?

There is a single simple and convincing explanation for all these strange relations; and it is one that throws a sharp light on the way gene mutations are produced. This explanation, originally due to Serebrovsky,[6] is as follows:

The numerous long thread-like chromosomes in the cells undergoing radiation lie close together and are more or less curved, coiled and tangled. They may cross each other and come in contact, but under ordinary conditions when this happens they do not stick together.

But under the action of radiation or high temperatures, the chromosomes change physically, in such a way that when they come in contact by crossing at a certain point they stick

together. There are indications (as Metz[7] has set forth) that the chromosomes have a transparent sheath, which keeps the essential parts of the chromosomes—the genes—within the sheath, from coming in contact. By radiations or heat this sheath is dissolved or modified in spots, so that the chromosomes within it may actually come in contact. When this occurs they stick together, and through strains set up in the intracellular processes they may break. The rest of the process will be best understood from an example (see Figure 12). Suppose that a chromosome in this adhesive condition becomes looped, so that its two limbs cross and come in contact (Figure 12, *A*).

The parts in contact stick together, and the chromosome breaks at the point of adhesion. The chromosome is thus left in three pieces, a central loop and two end pieces (Figure 12, *B*).

The three broken ends are still in contact, and there occur at the tips growth processes, so that the broken ends, some or all, may reunite. The two end pieces may reunite, leaving the loop free. In that way are produced the "deletions"; short chromosomes lacking all the middle part. In such cases the free loop degenerates or is lost.

In other cases the two end pieces may reunite with the ends of the loop. Sometimes the union may be with the same ends as before; then there might be no evidence that any break had occurred. But in other cases, since the four ends are all close together, each end piece may unite with the other end of the loop from that with which it was before united (Figure 12, *C*). This produces the inversions. The chromosome now has its two end parts as before, but in its middle part the order of the genes is reversed.

If the accidental crossing or contact is between two different chromosomes, then they adhere at the point of crossing,
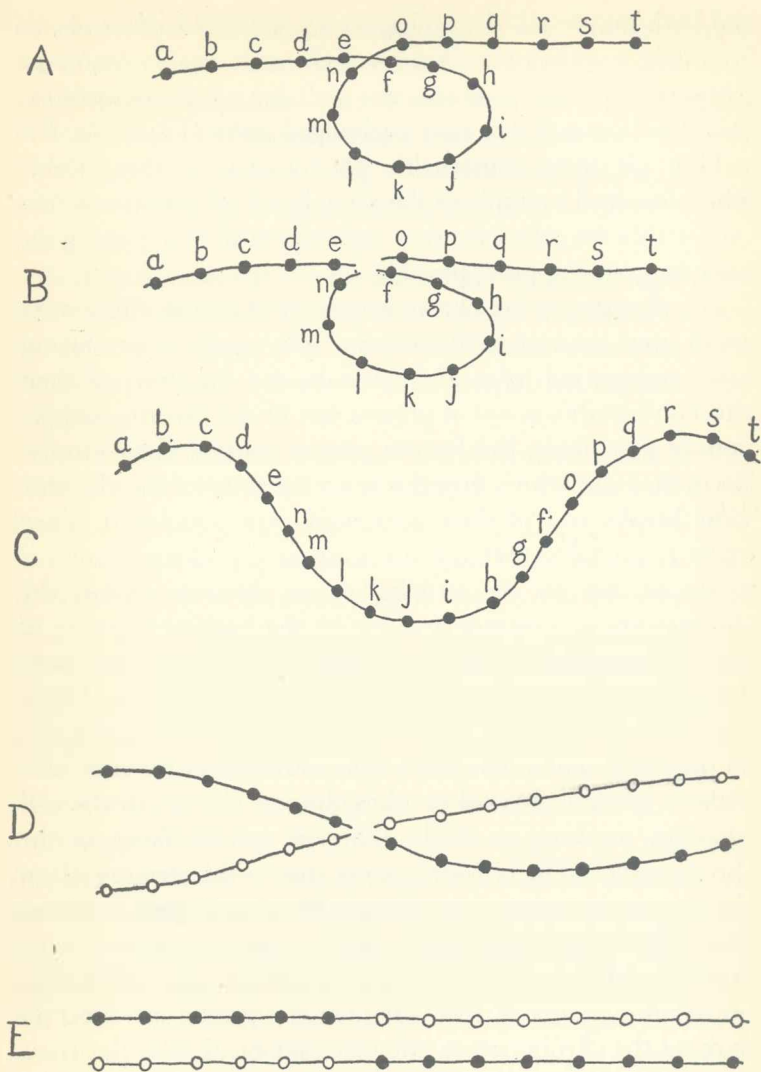
Figure 12. Diagrams of the method by which are produced inversions and translocations of the chromosomes and genes. See the description in the text.

and both may break there (Figure 12, *D*). Then the ends, in becoming reunited, may in some of the cases readily exchange partners. Thus are produced the well known translocations; two chromosomes that have exchanged parts (Figure 12, *E*).

Thus all these remarkable phenomena, so inexplicable otherwise, find a simple explanation based on conditions that exist within the cells. No other explanation than one along the lines suggested appears possible.

The changes in the chromosomes produced in these ways are of great interest in themselves. They result in producing new combinations of genic materials, and the study of their effect on heredity is just at present one of the favorite occupations of geneticists. But for our present purpose their interest lies in the light which they throw on the cause of the chromosome breaks and of their associated gene mutations. They make it probable that the breaking of the chromosomes is produced, not by the striking of an electron against the chromosome at a certain spot, but by the sticking together of the chromosomes. What the radiation does, what the heat does, is to modify or dissolve the chromosomal sheath. When, in this condition, parts of the chromosomes come in contact, all the rest follows. The rôle of the radiation or the heat is to make a general physical or physiological change in the cell contents, resulting in ready adhesion and breaking of the chromosomes. The breaking is not due to the striking of an electron at the point of breaking; this idea is quite inconsistent with the usual occurrence of two breaks in place of one, as well as with the fact that heat produces the same effect. The greater the energy of the radiation, the greater its effect in making the chromosomes adhesive and breakable; therefore the greater the number of breaks and injuries produced.

Important in this connection is the fact that breakage and the accompanying changes are not limited to chromosomes

that have been subjected to radiation or heat. Under natural conditions there occur translocations, inversions, and the other phenomena resulting from chromosome breakage, though much less frequently than under radiation. Variations in the physical state of the chromosomes, giving rise to adhesions and breakage, occurs at times under natural conditions.

And now come back to the mutations. Mutations, as before remarked, commonly accompany breaks, occurring at or near the point of breakage. The mutations must in some way result from the breaks, or from something that accompanies the breaks.

Just what is it in the breakage of chromosomes that brings about the changes called gene mutations? On the one hand there is the fact of breakage, the tearing apart of structures that were together, with its usual connotation of injury. The changed action of the genes might well be the expression of small scale injuries, induced at the same time as the greater injuries that are seen as breaks, deletions and deficiencies. The usually harmful or reductional effect of the mutations agrees well with this interpretation.

Another change that usually results from the breakage is an alteration in the relation of the genes to each other. When a chromosome breaks, the pieces usually reunite in a changed position, as we have seen, giving inversions or deletions or translocations. The result is that genes near the points of breakage are torn apart from those with which they are normally in close contact and are brought into close relations with other genes than those that are originally near them. It is suggested that possibly the effect that a gene produces depends on its position with relation to other genes; that is, presumably on the interaction of genes that are close together. Thus a gene moved to a new position with relation to others

produces an effect on development and characteristics that is different from its normal effect; and this changed effect is what has been called a gene mutation. This is spoken of as a "position effect." That the "mutations" which accompany chromosome breakage are such position effects is the view to which a number of recent investigators of these phenomena have come.[8]

What is certain is that when a gene is brought into a new position as a consequence of the occurrence of a chromosome break close to the point where it occurs, it often so changes its action as to produce what has been called a gene mutation. It is conceivable that the change in relative position is not the primary feature in producing the changed effect; it may merely provide the evidence of a break and mark the spot where it occurred. The change in effect would then be due to the lesions inseparable from laceration of tissues.

The nature of the changes in gene action that result from breakage have thus far been most fully studied by Schultz and Dobzhansky.[8] They examined thoroughly the effects on the genes in a case in which the second chromosome of Drosophila had been broken in two places, one at about the region of the gene bristle (54.7), the other near the gene brown (104.5) (see maps of the Drosophila chromosomes in any good text-book of genetics). That part of the chromosome between the breaks (nearly half its length) had been inverted and attached anew to the two end pieces, in the way illustrated in Figure 12, at *C*. At one of the breaks (that near *brown*), there was an effect on three of the genes. All three were weakened, so that they became unstable; in such a way that during development they went out of action in some of the cells, while in others they continued to operate, thus yielding mosaic individuals. In one of the three there was an additional, dominant, effect, showing itself in a change of the

color of the eye, which was altered from the usual red to a mosaic "plum" color. In the region of the other break two genes were altered. One, the gene "light," was made unstable, so as to yield mosaic organisms. The other was so altered that it would no longer support life unless there was present the other member of the pair in a normal condition (thus a "lethal mutation"). The fact that genes may thus be weakened by injury so that they go out of action capriciously during development is of great interest. The breakage in this case caused three types of effects on the genes: (1) a color mutation; (2) instability of certain genes; (3) a lethal effect on a certain gene.

The relation of chromosome breakage to gene mutations has received further unexpected illumination through the second of the recent discoveries mentioned earlier. Here we are dealing with things of the present moment; discoveries as yet hardly fully announced. In the flies, the organisms on which our detailed knowledge of genetics is so largely based, certain of the chromosomes become enormously enlarged, so that their complex constitution becomes conspicuously visible (see Figure 13). These are the chromosomes present in the salivary glands.

Each of these chromosomes is a large rod or thread, of dimensions in some cases about one hundred times as great as the corresponding chromosomes of other cells. Each shows a complex structure of bands and zones, differing in thickness and in physical structure. This structure is very different from that of the chromosomes as seen in the other cells of the body. For a long time it was not recognized that this elaborate structure in these salivary chromosomes has any relation to the structure of the chromosomes that has become known from their genetic effects.

But recently Painter[9] has discovered that the bands and zones of the salivary chromosomes mark the position of definite genes known from their effects in inheritance. In general, the banded structure corresponds to the known arrangement of genes and of mutations. In the normal flies, not mutated, the bands of any chromosome show a typical order and typical diversities in size and texture, as shown in Figure 13. In the different chromosomes of any species the banded structure differs; so that it is possible to recognize the particular chromosomes by this structure.

Study of the method of inheritance of the characteristics depending on known genes shows that in certain particular chromosomes a certain series of genes are reversed in their order, as compared with the usual arrangement (as in Figure 11). When this particular chromosome is studied as found in the salivary glands, it is discovered that in a certain region the order of the bands is correspondingly reversed. It thus becomes possible to know that the reversed genes are connected with the reversed bands. Again, in some cases a few genes are known, from genetic evidence, to have been lost from a certain chromosome; in such cases this chromosome, as found in the salivary gland, is found to have lost certain bands. By these and similar methods it has become possible to determine with certainty that the materials of particular genes are located in certain particular bands, having a definite position and structure. The great chromosomes of the salivary glands make clearly

Figure 13. The enlarged X-chromosome of Drosophila melanogaster as found in the salivary gland, after Painter, 1934.

visible certain relations that a few months ago one would have expected to remain forever hidden.

These great chromosomes, with their visible structure, provide a means of determining definitely whether certain chromosomes have been broken, with the production of deletions, inversions, translocations, and the like, for these things are clearly seen in the changed order of the bands. They make it possible to determine whether known gene mutations are accompanied by a break in the chromosome at or near the point where the mutation occurs.

This has already been determined for certain well known mutations. We have before seen that when a break is known to have occurred in a chromosome, this break is usually accompanied by a gene mutation. But many gene mutations have long been known with no corresponding knowledge of a break in the chromosome. Examination of the salivary chromosomes in stocks carrying such known mutations has shown for certain cases that the mutation is indeed accompanied by a break at or near the point of mutation; this break is presumably what gave rise to the mutation.

This has been demonstrated by H. J. Muller and his associates.[10] They have shown that a number of long-known mutations involve an inversion in the order of the bands of the corresponding salivary chromosome; including the band known to carry the material of the mutated gene. Such an inversion is known to be the result of chromosome breakage, as described on earlier pages. The inversions are found to be usually of small extent, often involving but two or three genes or chromomeres. It is of course important to remember that at the time the break and inversion occur, the chromosome is in the elongated, thread-like condition indicated in the diagrams of Figures 11 and 12; at such times inversions of small extent are readily possible. Such inversions of minute extent

are found to be unexpectedly frequent. In cases where the same gene has mutated in different ways in different cases (yielding what are known as multiple allelomorphs), the inversions are found to be visibly of somewhat different extent, or of different type, in the different cases.

An example of these relations, as described by Muller and his associates, is found in the mutations of the so-called scute gene. This is a gene that affects the bristles and their distribution on the body, in Drosophila. From genetic evidence it has long been known that this scute gene is at, or close to, the tip of the X-chromosome. This would bring it close to the first or second of the bands at the left or upper end of the salivary X-chromosome, as seen in Figure 13.

This gene scute has undergone many mutations—some twenty to thirty—each giving a slightly different effect. Now comes the essential point. In the stocks showing a mutation of the scute gene, there is found to be a change in the thick
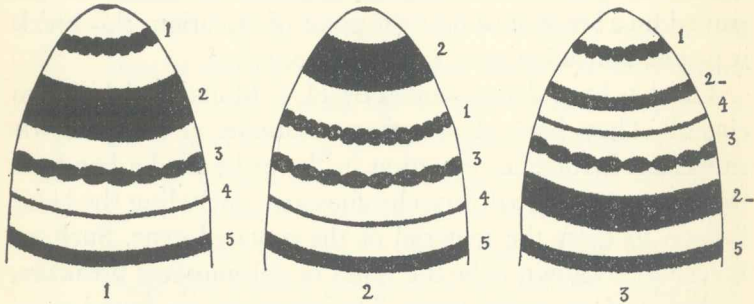
Figure 14. Diagrams of changes in the order of the bands in the salivary X-chromosome of Drosophila, in connection with mutations in the gene scute. *A, B* and *C* represent a portion of the extreme left or upper tip of the X-chromosome. *A.* The normal arrangement of the bands. *B.* Inversion of bands 1 and 2, in connection with one of the mutations. *C.* Rearrangement of the bands in connection with another mutation of the gene scute.

second band at the tip of the *X*-chromosome (see Figure 14);
a change sometimes affecting other bands near by, also. In
Figure 14, *A, B, C,* are shown diagrams, based on Muller's
description, of the changes in the bands in certain well known
mutations of the scute genes. Figure 14, *A,* shows the usual
condition in the normal chromosome, in which there has
been no mutation of the scute gene. Figure 14, *B,* shows the
situation in one of the well known mutations of this gene,
causing a change in the bristles. The first and second zones
have interchanged places. That is, the chromosome has been
broken close to the tip, and there has been an inversion of
small extent. (Besides the mutation in the scute gene, con-
nected with band 2, there was also another mutation, a lethal
one, probably due to the change in the gene connected with
band 1.)

Another mutation of this scute gene was accompanied by
an additional mutation in a gene close to it, affecting the
facets of the eye. In this case there had been a break and
inversion affecting bands 2, 3 and 4; the condition is shown in
our Figure 14, *C.* A piece has been broken out and inverted.
At the upper end the break has gone through the middle of
the thick second band, leaving part of it in place. At the
lower end the break was between bands 4 and 5. So the order
of the bands now is:

1— half of 2—4—3— other half of 2—5—6— etc.

In another mutation of the scute gene a part of the thick
second zone was found to have been broken out and attached
in another region of the chromosome.

These large salivary chromosomes are as a matter of fact the
two chromosomes of a pair united lengthwise, the corre-
sponding bands uniting throughout. Traces of the line
separating the two are observable, and in some cases the two

are separate for a short distance. It is not difficult to produce
individuals in which one of the two component chromosomes
is normal (without the mutation), while the other component
has the mutation and hence the changed arrangement of the
bands. These present a striking appearance.

Thus in these cases the mutation, long known, is shown
to be the result of a break in the chromosome; a break whose
occurrence is demonstrated by the changed order of the
bands. (Of course the break and rearrangement have
occurred when the chromosomes were in the extended condi-
tion indicated in Figures 11 and 12.)

In sum, it is clear that mutations are in many cases the
result of breakage of the chromosomes, with rearrangement
of some of the genes. In cases in which the first knowledge
is of the occurrence of breaks and rearrangements, these are
found to be accompanied by "gene mutations." And in cases
in which the first knowledge is that a mutation has occurred,
these are found to be accompanied by breaks and rearrange-
ments. Different mutations of the same gene are found to
result from the fact that the breakage occurs in different
regions of the chromosome, in different cases. The relation
of the so-called mutations to breaks and injuries is fully
demonstrated.

Certain further important questions at once arise. Are the
so-called gene mutations all of this type? Or are there two
classes, one occurring under the action of radiation and
resulting from breakage or injury to the chromosomes; the
other of a different type? Evidence on this will come soon,
from examination of the conditions in the salivary chromo-
somes, in the many stocks bearing diverse gene mutations. In
the meantime, the fact that similar and identical mutations
occur both under radiation and spontaneously, speaks strongly
against the existence of two classes differing in nature.

Again, are the mutations resulting from chromosome breakage essentially "position effects," or are they of the same type as deletions and deficiencies, save that they are of less extent or degree; injuries that do not result in complete destruction of genes, but only in that reduction in their effectiveness commonly seen in gene mutations?

If "gene mutations" should turn out to be simply position effects, this would require a rewriting of extensive chapters of genetics. In particular it would obviously have a devastating effect on the theory that these are the basic factors in evolutionary change. They would not be changes in the constitution of individual genes at all. They would be simply further illustrations of the known fact that the effects of a particular gene depend on the conditions under which it operates. A new class of such conditions is added to those before known; namely interaction with genes that are in close proximity within the chromosome. A gene removed from the normal grouping and brought into close relation with a new set of genes acts in a different manner from before; as the facts show, it commonly operates less efficiently, yielding reduced effects. The so-called multiple allelomorphs, supposed diverse modifications of the same gene, would be, so far as due to "mutation," simply the same gene in different positions with relation to other genes.

The general effect of such changes of gene action with change of position would be to increase the transformism and diversification of organisms by giving rise to many weakened and abnormal types, such as are commonly seen in mutated stocks. But their relation to progressiveness in evolution would completely evaporate. They would not supply the new genes with changed constitution that progressive evolution requires. They would have the same kind of relation to progressive evolution as have the other changes in the struc-

ture of the genetic system that were described in our second chapter.

Indeed, if this be the state of the case the history of these supposed gene mutations is but a continuation of the earlier history of supposed mutations, including most of the "mutations" of De Vries. These had a period of flourishing as the fundamental steps in evolution, but were later found to be only the result of regrouping and rearrangements among the chromosomes or parts of the chromosomes.[11] And now, if they turn out to be position effects the "gene mutations" of Morgan are found to be of the same type; they are rearrangements of small extent and often of complex character. They do not supply the material for constructive or progressive evolution.[12]

If on the other hand the mutations are rather injuries than position effects, the case for their rôle in progressive evolution is not better. In this case they are essentially disintegrative in character, and cannot be expected to give constructive or progressive results.

Taking the evidence all together, the case is strong for the essentially disintegrative nature of the observed gene mutations. It weighs heavily against their constructive or progressive character. It argues strongly against the possibility of explaining by them the adaptive and progressive features of the evolutionary process. The overwhelming majority of mutations, it is clear, are certainly not advantageous. Many of them cause death. Many cause a weakening of the constitution. Many produce obvious defects and abnormalities. Experimentation shows that most of them are reductional in their action, while those few that have positive action diverse from the normal are among the most distinctly injurious of mutations. And now, to complete the case, comes the demonstration that typical "gene mutations" are the result of breaks

and rearrangements in the genetic system; "position effects" or expressions of injury and disintegration.

Changes of this sort are not the material from which can be built progressive evolution. Their occurrence does not forward evolutionary progress, nay, it retards it. They do result in diversification, through the production of many abnormal and imperfect types. But with relation to progressive evolution, these are things to be gotten rid of. The mutations bring about the elimination of the individuals in which they occur or to which they are transmitted. They demand an enormous selective elimination that does not forward progressive evolution; at best it merely keeps evolution from going backward. When, if ever, the actual steps in progressive evolution are recognized as they occur, it appears probable that they will not be the result of action of destructive agents nor connected with disintegrative changes in the genetic system, but will rather bear a resemblance to the changes known as growth.

Whether these conclusions are sound will probably be fully shown within a few years, for it is clear that genetic science is on the threshold of an enormous development in relation to these matters. At the very least, it will be necessary in the future to make sharper distinction among the types of gene changes, not lumping together position effects and demonstrable injuries to the genetic system, along with constructive changes in the constitution of the genic materials.

In the meantime, in view of the formidable case against the observed types of mutation as supplying the fundamental basis for progressive evolution, students of these matters will be disposed to give more consideration to phenomena and conditions of other kinds, looking toward other types of genetic change as the basis for progressive evolution. No other

group of genetic changes is so well worked out as that of "gene mutations," but seemingly the more fully this is worked out, the less convincing becomes its rôle in evolution.

The efforts to find evidence of other types of genetic change fall into two classes. In one the evidence is indirect; through examination of the results of evolutionary change, conclusions are drawn as to its nature. In the other, genetic variations are directly observed as they occur in living organisms.

The indirect methods of obtaining evidence on the nature of evolutionary change may be classified in two main groups, with a third minor group.

I. In the first main group are attempts to trace the evolutionary changes that have occurred in the series of organisms that have lived in the past.

II. In the second group are studies of the diversities among organisms now existing, with attempts to judge of the nature of the changes that must have occurred in producing these diversities.

III. The minor group concerns changes that have apparently occurred in the genes of natural polyploids.

With these indirect methods of obtaining evidence on evolutionary change it is not the province of these pages to deal. Yet for comparison with the results of direct observation of genetic variations, certain typical lines of evidence may be sketched, with some of the conclusions that have been drawn from them.

I. To trace the evolutionary changes that have occurred in the series of organisms, from lower to higher, that have lived in successive geological ages, study must of course be limited to the hard parts of organisms, since only these are preserved. The literature of such study is of course enormous. As an example there may be mentioned the work of H. F. Osborn,

who has recently published a number of papers presenting conclusions drawn from the study of paleontological series.[13]

The conclusions of Osborn are based on his extensive studies of the fossil collections of the American Museum of Natural History, and elsewhere, the details of which are published in voluminous memoirs. His recent conclusions have been based largely on study of evolutionary series in the elephants and their relatives. Some of the essential data and conclusions may be sketched as follows:

1. Structures ("aristogenes") that in later ages become conspicuous and functionally important appear at the beginning as almost imperceptible features; so slight that their functional use is at this time doubtful. Classical examples of these things are the cones on the teeth of various vertebrates. They arise as almost imperceptible prominences (see the figures in the papers of Osborn above referred to).

2. In succeeding ages these structures increase in prominence and functional value in a continuous way without sudden changes. Continuity, not discontinuity, is the characteristic of such evolutionary change.

3. The structures follow a well marked definite course, in their changes from the imperceptible, doubtfully functional, condition, to the conspicuous and useful organs that they finally produce. Such definite change following a course toward a well defined useful end is spoken of at times as orthogenesis, or rectigradation.

4. In their later condition the structures produced are useful in a high degree.

5. The same types of structures (e.g. grinding cones on the teeth) arise independently in different divisions of the same group of organisms, so that they are said to be "predetermined in the germ plasm" of the group. Yet other divisions of the same group do not produce these structures, but others of a

different kind, so that the predetermination is such only in the
sense that the germ plasm is of a type that can produce these
structures if the conditions are favorable to them.

6. Such new structures as a rule take origin after there has
been a change of environment, or a change of habit on the
part of the organisms, of such a sort that the new structures
in time are useful and "meet the situation" under the new
conditions.

From these and similar data, Osborn draws a number of
important conclusions:

A. The new structures arise in response to a functional
need, in consequence of a changed environment or a change
of habit.

B. But they are not induced during the life of the individ-
ual, like the acquired immunity of an animal. They are not
direct adaptations of the individual animal to conditions, with
inheritance of these adaptations; not "inheritance of acquired
characters." Rather they are, as Osborn expresses it, "secular"
adaptations, requiring ages for their full production.

C. They are not "chance variations," or undirected varia-
tions, it is held, because they follow a definite course, toward
the fulfilment of a particular need. They are from the start
adaptive or "in the direction of future adaptations."

Attempts have been made, on the basis of the modern study
of mutations, to explain these phenomena in the following
way. The origin and farther advance of the new structures
are held to be in fact the result of undirected mutations;
mutations occurring with many diverse effects, as in Droso-
phila. The individuals in which these mutations are harmful,
or are not beneficial, in time die out or cease to propagate.
They therefore do not appear in the line of descent from the
early to the late stages of the given structure. But in some
cases the mutations happen to be useful. These continue to

propagate; and in some of their descendants again useful mutations occur, along with many that are not useful. Thus in the long run, there appear in the line of descent only those in which one useful mutation has followed another. Thus genetic variation appears to have followed a definite straight course; although as a matter of fact it has occurred in many directions, most of which were not useful and hence are not found in the pedigree of the final products. The fact that genetic variations do occur in directions that are useless or harmful is not in doubt, it may be pointed out, since useless and harmful mutations actually occur in the genetics of living organisms.

But in view of what we have seen of gene mutations in earlier pages, the question remains whether mutations indeed do supply the constructive and useful changes required for such evolutionary progress; or whether progress results from types of change not yet observed in living organisms.

II. The second of the indirect methods of studying the nature of evolutionary changes lies in the examination of the diversities among living organisms, attempting to judge how such differences must have arisen. On this matter there is of course a vast literature, dealing with taxonomy, anatomy, embryological development, geographical distribution and the like. Much of this was written before the rise of modern genetics, or by men not familiar with it, so that it is difficult to interpret in genetic terms. Other parts of it do not suffer from this difficulty. As an example of such work done in full awareness of the methods and results of genetics, and indeed making use of these, some features of the work of F. B. Sumner[14] may be sketched.

Sumner has made extensive studies of varieties and subspecies of certain wild mice—"deer mice"—of the genus Peromyscus; with attempts to judge of the nature of the differences

between them. The different types existing in nature have been
bred and intercrossed, to determine the method of inheritance
of the differences, and particularly, to discover whether these
differences behave like gene mutations. Some of the chief
results may be summarized as follows:

1. The differences between slightly diverse forms are in the
main heritable, not mere bodily effects of different envi-
ronments.

2. The slight differences between the different types—di-
verse varieties, subspecies or the like—are not like those due
to single mutations; they affect many diverse characteristics.

3. When the different types are crossed, the differences do
not behave like single gene mutations. They do not give sim-
ple Mendelian ratios, like those yielded when a mutant is
crossed with the parent from which it is derived. On the
contrary, the differences yield "blending inheritance," the
offspring being intermediate in most characters between the
two parent types.

"Blending inheritance" is known in many cases to be due
to the dependence of the characters so inherited on many
different genes, that differ in the two parents. This shows
therefore that the related types found in nature differ in many
of their genes; not in a single gene, as a mutant differs from
its parent form.

4. Hence if one of the varieties has arisen from another, or
if the two have arisen from a common ancestral type, there
have been slight genetic changes in many of their genes.

5. Such diverse types (varieties or subspecies) differing
slightly, but in many of the characteristics, commonly occupy
different, usually adjacent, geographical areas. Sometimes a
series of such varieties occupy a series of different areas.

6. Often in such cases there is a relation between the envi-
ronmental differences and the varietal differences, of the

following character. In a series of varieties occupying a series of zones, the series of zones show successive gradations of physical conditions, as climate, or soil, or elevation, from one extreme to the other; and there is commonly also a series of correlated gradations in the inherited characteristics of the varieties that occupy the successive zones. This appears to indicate that the physical conditions in some way affect the nature of the genetic variations; either directly, or by selective action on the variations produced.

On the whole, it appears that such series of varieties might have been produced through the gradual accumulation of slight germinal variations, under the selective action of the environmental conditions. But whether such germinal variations are mutations of the type observed in experimental genetics must be doubtful, in view of the evidence presented in earlier pages of this volume.

III. Further indirect evidence of changes in the germ plasm may be found in the conditions occurring in so-called polyploid organisms.

As was set forth in Chapter II, at times the number of sets of chromosomes, and therefore of genes, is increased. Most organisms have two sets, their chromosomes (and genes) being in pairs. Sometimes, through irregularities in the operation of the genetic system, the number of sets is increased to three, four, five, or more. Such organisms are called polyploids. In such cases the individual genes are likewise originally in threes, fours, or higher numbers, instead of in pairs. This changes the method of inheritance. There is, as before seen, strong evidence that some natural varieties differ in these ways; the number of sets of chromosomes has been increased to four, five, six or more. But in such natural polyploids as a rule there appear to be but two of each kind of gene, just as in other organisms. This shows that the genes in

some of the chromosomes have become altered, so that they no longer have the same effects as the corresponding genes in the other chromosomes of the set. The actual occurrence of such changes has not been observed, and their nature and causes are unknown. Possibly it is in such polyploids that the nature of evolutionary changes in the genes is most open for investigation.

In the direct attempts to observe genetic variations as they occur in living organisms, one direction that has long been followed lies in the relation of such variation to environmental action, and to the reactions of organisms to environmental conditions. In the lowest organisms, those made up of single cells, the occurrence of genetic variations has been observed on a large scale, and of these many seem more readily classified under the topic of environmental action and reaction than under that of gene mutation. An account of these will be given in the remaining chapters.

NOTES ON CHAPTER III

[1] Page 60. For a detailed account of such experiments see H. J. Muller, "Further Studies on the Nature and Causes of Gene Mutations," *Proceedings of the Sixth International Congress of Genetics*, Vol. 1, 1932, pp. 213-55 (see particularly pp. 231-52).

[2] Page 64. W. R. Horlacher and D. T. Killough, "Progressive Mutations Induced in Gossypium hirsutum by Radiations," *American Naturalist*, 1933, Vol. 67, pp. 532-8.

[3] Page 65. V. Jollos, "Weitere Untersuchungen über die experimentelle Auslösung erblicher Veränderungen bei Drosophila melanogaster," *Zeitschrift für Induktive Abstammungslehre*, 1932, Bd. 62, pp. 15-23.

[4] Page 65. See J. T. Patterson and H. J. Muller, "Are 'Progressive' Mutations Produced by *X*-Rays?," *Genetics*, 1930, Vol. 15, pp. 495-578; and further references there given.

[5] Page 68. See H. J. Muller, paper referred to in Note 1, and L. J. Stadler, "On the Genetic Nature of Induced Mutations in Plants," *Proceedings of the Sixth International Congress of Genetics*, Vol. 1, 1932, pp. 274-94.

[6] Page 71. A. S. Serebrovsky, "A General Scheme for the Origin of Mutations," *The American Naturalist*, 1929, Vol. 63, pp. 374-8.

[7] Page 72. C. W. Metz, "The Rôle of the 'Chromosome Sheath' in Mitosis and Its Possible Relation to the Phenomena of Mutation," *Proceedings of the National Academy of Sciences*, 1934, Vol. 20, pp. 159-63.

[8] Page 76. See Jack Schultz and Th. Dobzhansky, "The Relation of a Dominant Eye Color in Drosophila melanogaster to the Associated Chromosome Rearrangement," *Genetics*, 1934, Vol. 19, pp. 344-64. In this paper will be found references to other papers on this subject.

[9] Page 78. See Th. Painter, "Salivary Chromosomes and the Attack on the Gene," *Journal of Heredity*, 1934, Vol. 25, pp. 465-76; also other papers there referred to.

[10] Page 79. See H. J. Muller, A. Prokofyeva; and D. Raffel, "Apparent Gene Mutations, Due to the Position-Effect of Minute Gene Rearrangements," *Records of the Genetics Society of America*, No. 3, 1934, p. 48; also in *The American Naturalist*, 1935, Vol. 69, January-February, p. 72.

[11] Page 84. Among the so-called mutations of De Vries it has been the custom to distinguish certain ones as actual "gene mutations," while the rest are but rearrangements of chromosomes or parts of chromosomes. If the theory of position effects is substantiated, these "gene mutations" are likewise only rearrangements of parts of chromosomes.

[12] Page 84. This relation of the "position effects" to evolution is recognized by Muller and his associates. In the first publication giving an account of the relation of the scute mutations to the change of position of the zones on the salivary chromosome, Muller, Prokofyeva and Raffel (see Note 10 above) close with the following remarks:

"These and other cases raise the question: Are most, perhaps practically all, X-ray 'gene mutations' intergenic position effects rather than intragenic transformations? And the more momentous question follows: To what extent may natural mutations be of this type—a type incapable of providing the material for indefinitely continued evolution?"

[13] Page 87. See H. F. Osborn, "Aristogenesis, the Creative Principle in the Origin of Species," *The American Naturalist,* 1934, Vol. 68, pp. 193-233; and other references there given.

[14] Page 89. See F. B. Sumner, "Genetic, Distributional and Evolutionary Studies of the Subspecies of Deer Mice (Peromyscus)," *Bibliographia Genetica,* 1932, Vol. 9, pp. 1-106; also, "Taxonomic Distinctions Viewed in the Light of Genetics," *The American Naturalist,* 1934, Vol. 68, pp. 137-49.

CHAPTER IV

Genetic Variation in Unicellular Organisms, with Special Relation to the Inheritance of Environmental Effects and Reactions

THE remaining chapters will be devoted to some of the types of observed genetic variation in unicellular organisms; particularly certain types that do not fit well any of the categories of genetic variation thus far considered.[1] Genetic variations in unicellular organisms show relations to environmental conditions and to their reactions to these conditions, that are of interest for any general understanding of the nature of genetic variation.

Unicellular organisms differ from higher organisms in several obvious ways that are of great importance for genetic variation. First, genetic variation, in order to be effective, is not here restricted to a group of germ cells hidden away in the interior of a large body, as is the case in the higher organisms; for here the whole body is but one cell. Second, the entire organism is very small; in some of the unicellular organisms it is indeed so minute as to be beyond the reach of the ordinary microscope. In consequence, all of its materials are in close relation with the physical and chemical conditions of the environment, and exposed to their action. And third, parent and offspring are not separated by a long series of cell generations. In the higher organisms, the offspring begin as single cells, and a long and complex process of development occurs, involving thousands or millions of cell divisions, before the adult condition is reached. All this is lacking in unicellular organisms. Reproduction here occurs by a single

division of the parental cell, each of the two new cells pro-
duced transforming into an adult individual. Thus the one-
cell organisms show us the nature of heredity and variation
as these occur when there is but a single cell division between
parent and offspring.

Yet it is important to realize that even in this reproduction
by a single division, the individuals of the new generation are
produced by development. Even the unicellular organisms
are complex systems of structures; they are small machines.
We cannot multiply machines merely by cutting them in
two; try that on a typewriter or a gasoline engine! If the
organism is simply divided, the unity and harmony of its
machine-like structures are lost. During division many or
most of the definite organs disappear, by absorption or disso-
lution, and are then after division produced anew; they are
not transferred bodily from parent to offspring. This is true
of all the unicellular organisms, even the simplest, but it is
best seen in the more complex types. Some of the infusoria
bear on their bodies numerous differentiated appendages
serving as organs of locomotion; these are arranged on the
body in a definite pattern. When the animal divides, these
appendages disappear, and as the body separates into two
parts a new set of appendages appears and develops separately
on each half, finally arranging themselves in the typical
pattern (Figure 15). Thus inheritance of the organs and
pattern is not direct transfer from parent to offspring, but is
new production of structures like those which the parent pos-
sessed. Yet even so, reproduction by a single cell division is
relatively simple in comparison with the long process of
development with many successive cell divisions, that occurs
in higher organisms. To this difference is perhaps to be
attributed some of the peculiarities of heredity and variation
in these organisms.

When we examine unicellular organisms, we find that the individuals of a species differ among themselves, just as is true in higher organisms. If one collects a large number of individuals of some unicellular animal—say Paramecium, or
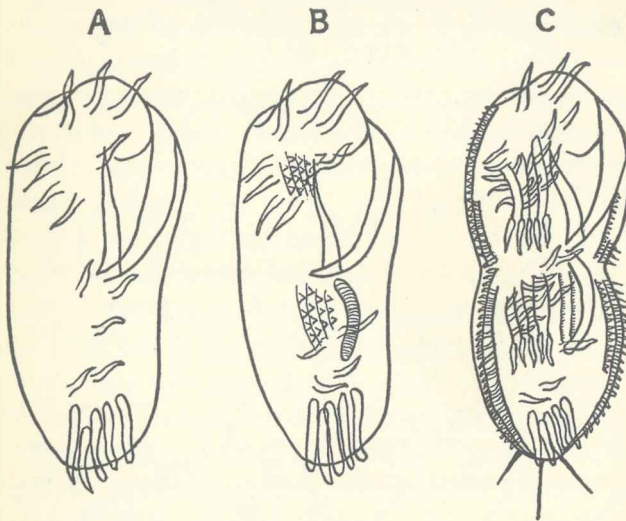

Figure 15. Reproduction and development in the infusorian Stylony-chia, after Wallengren, 1901. *A.* Parent before division, showing the number and distribution of the locomotor appendages. *B.* Beginning of division: appearance of two groups of small projections, that are later to form by enlargement and distribution the appendages of the two offspring. *C.* Later stage of division: the two groups of embryonic appendages are scattering to take their final positions. The old appendages have not yet disappeared.

Difflugia, or Arcella—one usually finds diversities of many kinds among them. In the shelled rhizopod Difflugia corona (Figure 16), an animal closely related to Amoeba, one finds the individuals differing greatly in size, form and structure of their shells, as well as in many physiological ways. Many

of these differences are inherited; when the individuals repro-
duce, their descendants show characteristics like those of their
parents. Again, in the well known infusorian Paramecium,
one finds a large number of diverse stocks, differing in size,
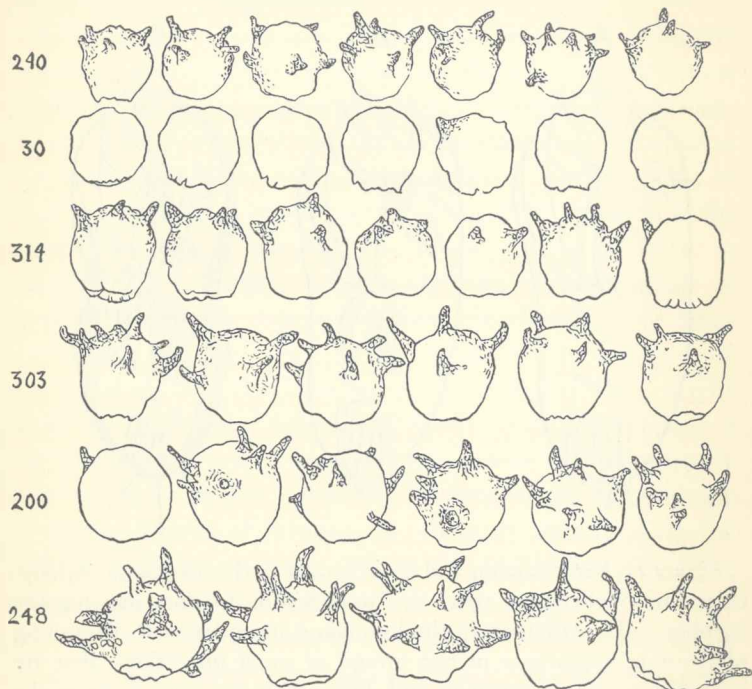
Figure 16. Inherited differences in Difflugia corona. Six races or
biotypes differing in size, number and length of the spines, and in
other respects. Each horizontal row is a series of individuals all derived
by successive divisions from the individual at the left end of the row.
After Jennings, 1916.

and in other respects. Within each stock there are size differ-
ences due to diversity of age and nutrition; but the mean
differences are inherited; they are handed on from generation
to generation.

Such inherited diversities one finds in any unicellular organism, if it is studied with sufficient thoroughness. And there are similar variations in less conspicuous matters. There are differences in vigor, resistance, vitality. Under poor conditions, some races die quickly, others resist long. Some are adapted to one set of conditions, some to another. Some stand higher temperatures than do others. Some are resistant to a chemical to which others quickly succumb. Some multiply rapidly, others slowly. Some races of Paramecium conjugate frequently, others rarely. Differences of all these types are inherited.

What is the origin of these inherited diversities? This is the problem of genetic variation.

In the unicellular organisms, as in those composed of many cells, several different types of genetic variation occur, some of them showing direct correlations with environmental conditions such as have not been demonstrated in higher organisms. It is these that are of special interest for our present purposes. But before examining these, certain other types of genetic variation must be briefly presented.

In the unicellular organisms, as in higher organisms, biparental reproduction is a source of genetic variation. In such ciliate infusoria as Paramecium, at times two individuals unite temporarily in conjugation and exchange parts of their nuclei, then separate and continue reproduction by fission. Conjugation among the members of a uniform stock produces among the descendants races or stocks differing greatly in their inherited characteristics. They differ in size and in other physical features. This has been shown by the recent work of Sonneborn and Lynch, and by that of De Garis. Thus, De Garis produced repeated conjugations between the members of a uniform stock of Paramecium caudatum, the individuals of which, when full grown, had a mean length of 172

microns (De Garis' race 75). From four pairs of this race
there were produced four stocks showing respectively the
following mean sizes: 165 microns, 133 microns, 184 microns,
136 microns. From his race 3, having a mean length of 198
microns, there were two pairs, giving rise to two stocks of
having respectively the mean lengths of 70 microns and 203
microns. From two ex-conjugant pairs of race 17, having a
mean length of 153 microns, were produced two stocks of the
following mean sizes: 145 microns, 170 microns. Again, when
two races of diverse mean size are repeatedly crossed, stocks of
diverse sizes are produced. De Garis made several crosses of
his race 39, of 203 microns mean length, with members of his
race 11, of 81 microns mean length. Four pairs gave stocks of
the following mean lengths, respectively: 144 microns, 191 mi-
crons, 207 microns, 91 microns. Crosses between the race 56
(mean length 191 microns) and race 83 (mean length 75
microns) gave from three pairs stocks of the following mean
lengths, respectively: 65 microns, 140 microns, 111 microns.

The different stocks produced by conjugation within a
single uniform stock differ greatly in vigor, in rate of multi-
plication, and in resistance to unfavorable condition. This
has been conspicuous in the investigations of myself and
associates on Paramecium aurelia and Paramecium caudatum.

This production of inherited diversities at conjugation is
presumably due to the fact that conjugation, like biparental
reproduction in higher forms, is a method of forming many
diverse combinations of genes or genic substances. The indi-
viduals having the different combinations show very different
characteristics, as to size, fission rate, vigor and resistance.

Furthermore, it can be shown that even during reproduc-
tion by fission there may occur at times irregularities of
division that result finally in the production and isolation of
diverse stocks, differing in hereditary characteristics. For by

long-continued careful selection of differing individuals throughout many generations, in a stock that is multiplying by fission, inherited diversities may be produced. It is true that such selection may be practised for long periods with no apparent results; but if it is persisted in for a very long time, and a suitable basis for selection is employed, there may finally result inherited differences. In Difflugia corona there were produced in this way, in my own work, races slightly diverse in form and size. Middleton and Parker found that by long-continued selection in infusoria, races differing in inherited rate of multiplication could be isolated.

Just what processes underlie these changes is not known. It appears possible that there occur at times irregularities of nuclear division, analogous to some of those described in Chapter II for higher organisms, resulting in unequal distribution of the genic materials in the two individuals produced, so that they have diverse inherited characteristics. The inherited differences produced in this way have thus far been but slight.

In addition to these genetic variations resulting from the production of new combinations of genic materials in biparental reproduction, and those presumably due to irregularities in nuclear division, there are others that occur under the action of environmental conditions, and that show special relations to the conditions that induce them. These are of special interest; to them we now turn our attention.

It is easy to change the characteristics manifested by unicellular organisms, by subjecting them to changed environments. In Difflugia the size, form, number of spines, and length of the spines are readily altered by subjecting the animals to certain conditions. By subjecting Paramecium to certain chemicals, they can be altered greatly, in size, form,

rate of multiplication, and in other respects. Other Protozoa can be readily altered in similar ways.

But usually, when the animals are restored to the original conditions, in the offspring there produced the same old form, structure and functioning return. I have myself made extensive experimental studies on this, in Paramecium and in Difflugia, by subjecting them to diverse chemical and other agents, which changed them greatly. But on removal from the chemical, in the next generation the animals returned to their original state; the acquired characters were not inherited. This is the common result of such experiments. The substances of the organisms have not been modified in constitution; it is only the superficial characteristics that have been changed. The effects are like those of changing the shape of a crystal, by filing off its angles or carving it into new shapes. If such changed crystals are dissolved and recrystallized, the original crystal form returns, for the chemical constitution has not been changed.

Most effects of the environment are of this kind. They do not change the constitution of the fundamental materials of the organism; therefore they produce no genetic variation; no lasting effect on its characteristics.

But in the unicellular organisms, not all environmental action is of this sort. Sometimes, by environmental action, the underlying substances *are* changed in constitution, so that a genetic variation is induced, a variation that is carried on to later generations.

The difference in effect appears to be connected with the length of time that the effective agent continues to act. If the organisms are subjected for generation after generation successively to the special conditions, so that these act for a very long time, then the constitution may become changed; there is produced an alteration in the inherited characteristics, a

change that is passed on to later generations. This factor of
time, or of something connected with the time, of action,
appears of great interest.

In unicellular organisms such results have been produced,
not by one or two external agents only, but by many different
types of environmental conditions; by conditions that operate
on the organisms in their normal life. Many different types
of inherited alterations have thus been produced. Some of
them are degenerative; they leave the organism the worse for
their action. Others are distinctly to the advantage of the
organism. They are what can be called adaptive changes in
the hereditary constitution. Changes produced by a particular
environmental agent may have a definite specific relation to
the agent that produces them, fitting the organism to survive
and thrive under the action of that agent. We shall examine
here the main different types.

The first type of inherited environmental effect brought to
light consisted of inherited injuries to the constitution of the
organism, weakening it, inducing a degenerate condition. It
has often been suggested that in man, life for many genera-
tions under evil conditions may produce inherited degenera-
tion. In unicellular organisms this is indeed true. It was dis-
covered through the classical work on life histories, depres-
sion, degeneration and rejuvenescence in Protozoa, begun
fifty to sixty years ago by Balbiani and Maupas, and continued
by many investigators from that time to this.

Everyone who has had experience in this kind of work
knows that if one cultivates an infusorian for generation after
generation in isolation, on glass slides, or under other condi-
tions differing much from nature, as a rule the organisms
begin after a long time to "run down." They divide less fre-
quently, they do not feed well; they lose resistance to bad
conditions; they have digestive and assimilative troubles;

they show unfavorable symptoms of many kinds. The prog-
ress of these changes is well shown by plotting a curve of the
frequency of division of the animals (Figure 17). This curve
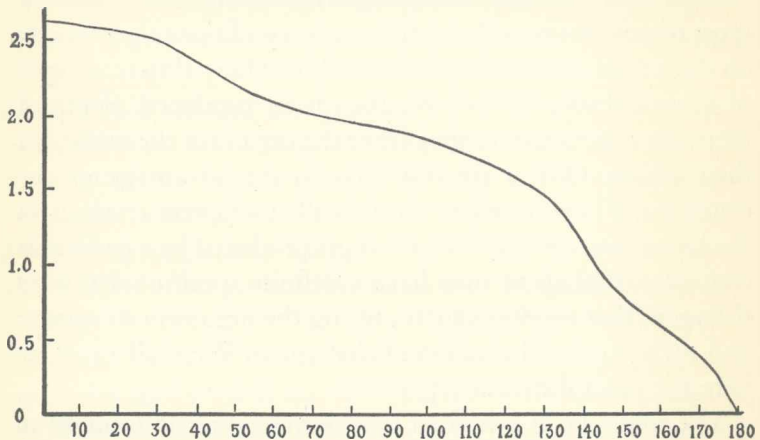of the rate of reproduction gradually "runs down hill,"

Figure 17. Partly smoothed curve showing the gradual decline in
the rate of reproduction in Stylonychia pustulata, when subjected to
a high temperature (28 to 32 degrees). The vertical scale shows the
mean number of fissions per day; the horizontal scale shows the num-
ber of days from the beginning of the high temperature. Constructed
from the data of Middleton, 1918.

though with various fluctuations. There is on the whole a
rather steady downward progress, sometimes to a final cessa-
tion of reproduction, and death of all the organisms. The
slowing of reproduction is accompanied by degenerative
changes in the other functions above mentioned. The degen-
erative changes are not perceptible till many generations have
elapsed, and many more generations are required for the slow
descent to deep depression or death.

As to the exact nature of the changes that are occurring,
there have been differences of opinion. Some have believed

them to be in essence of the same sort that occurs in growing old, in the case of higher organisms. Others think of them rather as damage to the constitution resulting from bad living conditions. The essential facts for our purposes are, first, that the changes are alterations in the hereditary constitution; in the basic materials of the organism. And second, that the environmental conditions play an important rôle in bringing on these changes. In some species, indeed, if not in all, a bad environment is the exclusive factor in bringing about the degenerative changes; they do not occur if the animals are kept in a favorable environment, but do occur if the conditions are not quite those fitted to the organism. Such decline of vitality, or depression, may be produced by subjecting the animals for many generations to unfit food, to scanty food, to too high a temperature, to the action of various chemicals, and to various other unfavorable conditions.

Thus the material of these depressed organisms has in the course of many generations become gradually altered, depressed, damaged. But what is of the greatest interest, this damaged material continues to assimilate, continues to produce more of its own damaged material; it grows and reproduces in the damaged condition, producing new generations showing the depressed characters. For if we compare side by side under the same conditions a stock that has been under bad conditions with one that has not, it is found that both reproduce, both multiply, but the difference between them continues. Under the same conditions one multiplies rapidly, at a high level of vigor, with high resistance, and good digestion and assimilation; the other slowly, at a low level, with poor resistance, poor digestion and assimilation. This may continue for twenty, fifty, one hundred, five hundred generations or more. While the materials of the normal individuals multiply themselves in the normal condition, the

damaged materials of the depressed individuals multiply themselves in the damaged condition. In merely ten generations the damaged material has produced more than a thousand times its original amount; in a hundred generations it has multiplied itself by billions.

Thus in these unicellular organisms, the condition of the individuals of the present generation as to health and vitality depends on the conditions in which their ancestors have lived; it may depend on the ancestral environment a hundred generations ago. If their ancestors a hundred generations back have been living for many generations under bad conditions, the present individuals are weak and depressed. If their ancestors of a hundred generations ago have been living for many generations under good conditions the present generation is strong and vigorous. We have then in these organisms the "inheritance of acquired characters"; the inheritance of environmental effects, at least insofar as damage to the genetic constitution through bad living conditions is concerned.

But such inheritance is not limited to injurious effects of the environment. It occurs too when the action of the environment is such as to change the organisms for the better. The environment may produce in unicellular organisms changes that are adaptations to the conditions that produce the changes. When subjected for long periods—for hundreds or thousands of generations—to gradually increasing temperatures, infusoria may slowly acquire an increased resistance to high temperatures. The increase may be very great; Dallinger and Drysdale in seven years increased the temperature which certain species of infusoria could resist, from about 25 degrees centigrade up to 70 degrees; or expressed on the Fahrenheit scale, from about 80 degrees to 150 degrees. Before the experiments, none of the organisms could endure these high temperatures; at the end all live and thrive in such conditions.

Also, if certain Protozoa are subjected for long periods to solutions of certain chemicals, their resistance to these chemicals may after many generations become increased. Such a result, it may be remarked, is not readily and easily reached; many investigators who have attempted to produce it have failed. But with some Protozoa, under proper conditions, striking results are reached; the resistance is greatly increased. The animals become acclimatized or immunized to different chemicals, just as higher animals become immunized to certain organic poisons and bacterial products, so that they can later live and thrive under conditions which earlier would have killed them. In these respects the relations are the same in unicellular organisms and in higher animals, save that in the unicellular forms many generations are required for the production of immunity.

These matters have been studied most fully in the bacteria and in the parasitic Protozoa.[2] In the trypanosomes, flagellate Protozoa that live in the blood of various mammals, including man, the relations found are these: When the vertebrate host —for example, the rat—is infected, the parasites multiply rapidly for a time by fission, till very large numbers are present in the blood. After a time, almost all of these suddenly die. This is due to the production by the host of an "antibody," which is poisonous to the trypanosomes. But not all the parasites are killed, for later a large number of them reappear, inducing a "relapse." Investigation shows that this recrudescence of the parasites is not due to the disappearance of the antibody from the blood; it is still present and it is still fatal to parasites of the type that were originally present. What has happened is that a small proportion of the trypanosomes have acquired resistance to the antibody; they can now live in its presence. These immunized individuals then multiply, so that the host again becomes filled with parasites,

now resistant to the antibody. This has been found to occur even when the original infection was by means of a single trypanosome, so that it is clear that resistance has been acquired by individuals of the same strain that were before non-resistant. In a similar way, resistance has been found to be induced toward drugs introduced into the body for the purpose of killing the parasites.

The resistance once acquired lasts for a long time, for hundreds or thousands of generations. Ehrlich found that resistance thus induced by the arsenious drug atoxyl continued for three years, even though the parasites had been transferred to animals in which the drug was not present.

Great numbers of cases of this type are known in the parasitic Protozoa. This acquisition of new and adaptive features, inherited in reproduction by fission, is a characteristic feature in the life of these parasitic organisms; it is a matter of great practical importance in the treatment of the disorders caused by the parasites.

Knowledge of these matters in the free-living Protozoa is less extensive; there is much need here for further work. But a number of the features that are of importance for genetics have been brought to light, principally by the work of Jollos, and of Neuschloss. By long subjection of the free-living protozoan Paramecium caudatum to certain chemicals, resistance to these chemicals has been greatly increased. This has been accomplished with certain compounds of arsenic and antimony, and with certain organic compounds, notably quinine, methylene blue, trypan blue, and fuchsin.

It was shown by Neuschloss that the acquirement of resistance to such materials is due to an induced change in the intimate chemical processes occurring in the animals; a change in their metabolism. All the substances named above are injurious to the organisms. The Paramecia acquire

through long subjection to a weak solution of them a power to transform the harmful substance into something else that is innocuous; practically to destroy it. It was found that each of the above named organic substances induces a different change in the animals, so that infusoria that have acquired the power to resist and destroy quinine do not resist and destroy methylene blue, and vice versa; and similarly for the other substances. The acquired resistance is specific for each substance. Thus the materials of which the organism is composed —or certain of these materials, at least—are changed in a different way by each of the chemicals to which resistance is acquired.

But what is most important for our purposes, such acquired resistance is inherited, in the free-living Protozoa as well as in the parasitic ones. The organisms are removed from the chemical that has induced the resistance. They continue to multiply by fission, and their descendants for hundreds of generations continue to possess the resistance acquired by their ancestors. In the course of a great number of generations under conditions lacking the chemical, the increased resistance may gradually decrease and finally disappear; to this fact we return later.

But the positive fact of inheritance for hundreds of generations shows clearly that the organisms, or certain material of the organisms, have become changed under the action of external conditions, and that this modified material continues to assimilate, increase, reproduce, in its modified condition. This modified, more resistant substance increases itself a thousandfold in ten generations; many millions of times in a hundred generations. It is clear therefore that the environment has produced a genetic variation; an inherited variation.

Such changes are particularly striking in the bacteria; here they are of great practical importance, as well as of great interest for the general properties of living matter. The bacteria are perhaps the lowest organisms for which we can hope to have a knowledge of genetics and variation. They are extremely minute; certain of the common forms are spheres about one micron, or one-thousandth of a millimeter in diameter. Each has the volume of less than one cubic micron. Taking the volume at that figure, calculation shows that it requires fifteen thousand billion of such bacteria to yield the volume of a cubic inch. Thus all parts of the bacterial body are in extremely close relation to the surrounding conditions; all parts are within half a micron, or one fifty-thousandth of an inch, of the enveloping conditions, chemical and physical. There is no part of the bacterial body that is protected from the action of the surroundings by being lodged in the interior of a large body, as is the case in higher organisms. This is a great and important difference.

We know that the minute bacterial body is the seat of chemical activity of a high potency, manifesting itself as fermentations, putrefactions, production of diseases and the like. All this is occurring in closest contact with surrounding conditions. Reproduction occurs by division of the body into two. When this division occurs under uniform and favorable conditions, usually the two parts produced have the same constitution and characteristics as the parent, and this continues indefinitely, so that the stock remains uniform, without genetic variations. If we have side by side two different types of bacteria, when they divide the diversity continues; the characteristics of each type are inherited; the two types remain constant. For many years there was an effort to show that this is always the case in bacterial reproduction. The uniform

and favorable kinds of culture media commonly employed seem to have favored this effort; under the conditions supplied there was little genetic variation.

Yet even so, many phenomena were observed that did not agree with this interpretation. In a supposedly pure culture of a single type, there appeared types that were diverse in form, structure or physiology. It was believed that in most cases such phenomena were due to contamination. The supposedly pure culture was held not to be pure. Mingled with the prevailing type were presumably a few of another type, undetected. Under certain conditions these did not multiply, so that they remained inconspicuous. But under changed conditions these hidden bacteria multiplied and became prominent. Thus there appeared to have been a genetic variation, a change of type that was inherited. But it was held that all that had really happened was the substitution of one type for another.

There were, however, certain changes, particularly in disease-producing bacteria that were very extensively studied, which could not be explained in that way. In old cultures, or under changed conditions, the forms, structures and physiological action of the cells were greatly altered, and these changes persisted even when the organisms were returned to the original conditions, so that genetic variation had occurred. These were characterized as abnormal or involution forms, and their significance therefore slighted; although for the problems of genetic variation such changes are of high interest.

In recent years these changes have been studied systematically and thoroughly for their own interest. The effects of changed environmental conditions have been extensively studied. Improved methods have been devised, for beginning a culture with a single bacterial cell, so that apparent changes

that occurred could not be accounted for as due to contamination with another bacterial species.

The result of these intensive studies has been to show that genetic variations of many types do indeed occur in bacteria, and particularly that such are brought about by changed environmental conditions. They show that many well known changes in bacterial cultures are due, not to contamination, but to inherited changes produced under the action of the environment.[3] Some of the commoner types of change are the following:

Attenuation, or decrease in virulence: Disease-producing bacteria are kept for a long time under unfavorable conditions, and these cause them gradually to lose their power to produce disease. This decreased virulence is hereditary.

Increase of virulence: A change, the reverse of that just mentioned, is brought about in many disease-producing bacteria by subjecting them to appropriate conditions. By inoculating them successively into animals of higher and higher resistance, they are caused to acquire by degrees a higher virulence, and this acquired virulence is inherited.

Another type of inherited change induced by environmental conditions consists in alterations in the secretions or excretions of the bacteria. Bacteria that secrete mucous coverings are caused to secrete less mucus, or none at all, and the changed condition is inherited. Some bacteria secrete colored materials; by subjection to certain conditions, they are caused to cease producing the colors; and the change is inherited.

Most important perhaps are the acquirement of adaptive changes in metabolism. There are certain kinds of bacteria that can split up particular kinds of sugar, such as lactose, getting nutriment from them. Certain other related races cannot. If cultivated in the presence of this kind of sugar, these

latter make no use of it, for several or many generations. But in the course of time some of them develop the power to split up this sugar and to thus obtain nutriment from it. They thereupon begin to multiply and flourish greatly. And such individuals transmit this newly acquired power to their descendants for a great number of generations, even though the descendants may live where there is none of the sugar that induced the change.

Many examples of this type of change could be cited; and many other phenomena of extraordinary interest for genetic variation are known in the bacteria. Instead of being patterns of uniformity and constancy, as was supposed, it is now found that bacteria develop genetic variations, inherited changes, with great frequency and in great variety. The technical worker who endeavors to keep his conditions constant by employing cultures of a single strain of bacteria is dismayed to find that his single strain has broken into two or more, with diverse physiological properties. Particularly are the bacteria genetically sensitive to changed environments; these induce new inherited characteristics; so that the "inheritance of acquired characteristics" is a characteristic feature of genetics in these organisms.

It must be said that the phenomena of genetics and genetic variation are by no means fully worked out in the bacteria. At present there is much difference of interpretation as to some of the phenomena. In some quarters we read of free bacterial genes that circulate about in nature and that may become associated with various bacterial stocks, changing their heritable characteristics; and other adventurous views are put forth.[4] When these matters are fully cleared up, they will beyond doubt yield a most important chapter in genetic science, and particularly in genetic variation. In any case, the

great rôle of environmental conditions in producing inherited variations is not in doubt.

Thus in the unicellular organisms in general—bacteria and Protozoa—we find that environmental conditions may produce genetic variations of many sorts. We may classify them under two heads. On the one hand they may produce injury. The materials of the organisms are damaged, but continue to multiply in the damaged condition, so that a long series of depressed or degenerate generations is produced. On the other hand, the environment may produce adaptations; it may result, not in damage, but improvement. The organism under the influence of the environment acquires power to do certain things that it could not before do; to bring about chemical reactions of which it was before incapable, and so to live under conditions under which it formerly could not live. These acquired powers are inherited for many generations after removal from the conditions which induced them.

In producing such results, there are certain important relations between the length of time that the special environmental condition has acted, and the intensity and duration in generations of the genetic variation that it produces. In many cases the degree or intensity of the inherited effect is proportional to the length of time that the effective environmental condition acts. This is notably the case with the inherited damage resulting from living under bad conditions; the damage does not appreciably begin till the conditions have existed for many generations; it increases in amount as the bad conditions last longer, and finally may result in destruction of the stock. The constitution is progressively more modified the longer the conditions last. The same relations are found in many cases in the production of inherited resistance by the

action of heat or chemicals; the longer the action, the greater
the resistance produced. The action of the environmental
conditions on the materials of the organism is progressive.

There is in many cases a similar relation between the length
of time the effective agent acts, and the length of time meas-
ured in generations, that the induced genetic variation con-
tinues to be inherited, after removal from the conditions
that produce it. When the environmental condition—the heat
or the special chemical or the like—has acted but a short
time, usually its effect is not inherited at all. The constitution
has not been modified. If, however, the special environment
has operated for a longer period, its effect is inherited for a
number of generations, but finally disappears. If the action of
the special environment has continued for a very long period,
through many generations, the inherited effect continues for
a great number of generations after removal from the condi-
tions that induce it. An example of these relations is seen in
the inheritance of an abnormality in the shell of the rhizopod
Arcella, as studied by Jollos. The abnormal form of the shell,
known as double-lobing, is partly produced by the accumula-
tion of waste products in the fluid in which the animals
live. If after the abnormality has persisted for many genera-
tions, the individuals that are least abnormal are continually
selected for propagation, gradually the population becomes
normal. When the animals had lived for four months in the
condition tending to produce the abnormality, it required
two months to bring them back to normal; when they had
lived for seven and one-half months in such conditions it
required three months to bring them back to normal; when
they had lived for twelve months in such conditions, four
months were required to bring them back to normal. In these
cases there was about one generation or more a day, so that

the number of generations in which the abnormality was inherited was very great.[5]

Thus the action of the environmental conditions on the genic substances is gradual and progressive. The longer the environmental condition acts the greater is the effect produced. And the longer the environmental condition acts, the longer does the acquired character persist; that is, the greater is the number of generations through which it is inherited, after removal from the conditions that induced it. The modified organic substance multiplies itself a millionfold. But finally, as a rule, if the organisms live sufficiently long in an environment that does not contain the effective condition, it loses the acquired character. Thus under new conditions, new inherited characters are acquired, but only after many generations. On restoration to the old conditions, the old characteristics are restored, but again only after many generations.

In a general view, therefore, we find that the condition and capabilities of the unicellular individual depend on the way its ancestors have lived. If the ancestors have lived for many generations under conditions that gradually injure their genic substances, the injury is handed on to the children, not merely to the third or fourth generation, but to the hundredth or thousandth generation, causing them to be weak and degenerate. If the parents have lived in such conditions as to acquire new powers, these new powers too are handed on to the hundredth generation and later. Their welfare and capabilities depend largely on how their ancestors have lived. The general situation appears to be that which is implied by the terms "Lamarckian inheritance," and "the inheritance of acquired characters."

Lamarckian inheritance has long been one of the great problems of biology; no subject in this field has been more disputed. One might say that all theory has been for it, all

experience against it. The observed occurrence of such inheritance in the lower organisms is of great interest. It is worthy of careful examination. In the next chapter we shall undertake such an examination. We shall inquire into the processes that underlie these changes, and their significance for the general problems of evolution.

NOTES ON CHAPTER IV

[1] Page 95. For an account of our knowledge of the genetics of unicellular animals up to 1929, see the present author's "Genetics of the Protozoa," *Bibliographia Genetica,* 1929, Vol. 5, pp. 105-330. In this work will be found an extensive bibliography. References there given will not be repeated in these pages. For detailed accounts of investigations briefly referred to herein, reference may be made to the above work. Only in the case of investigations made since 1929 will reference be made to the original papers.

[2] Page 107. For a detailed account of the conditions in some parasitic Protozoa, see W. H. Taliaferro, "Host Resistance and Types of Infections in Trypanosomiasis and Malaria," *Quarterly Review of Biology,* 1926, Vol. 1, pp. 246-69. See also by the same author, *The Immunology of Parasitic Infections,* 414 pp., New York, 1929.

[3] Page 112. For an extensive review and discussion of the variations in bacteria under the action of the environmental conditions, see Philip Hadley, "Microbic Dissociation. The Instability of Bacterial Species with Special Reference to Active Dissociation and Transmissible Autolysis," *Journal of Infectious Diseases,* 1927, Vol. 40, pp. 1-312.

[4] Page 113. See the recent review by W. H. Manwaring, "Environmental Transformation of Bacteria," *Science,* 1934, Vol. 79, pp. 466-70.

[5] Page 116. A somewhat detailed and illustrated account of this work, with references to the original, is given in *Genetics of the Protozoa* (Note 1, above), pp. 261-8.

CHAPTER V

GENETIC VARIATION IN UNICELLULAR ORGANISMS, CONTINUED.
WHAT IS THE METHOD OF OPERATION OF THE GENETIC SYSTEM
IN BRINGING ABOUT GENETIC VARIATIONS UNDER THE LONG-
CONTINUED ACTION OF THE ENVIRONMENTAL CONDITIONS?

THE preceding chapter set forth that in unicellular organisms, changed environmental conditions eventually cause corresponding changes in the characteristics; and these changed characteristics may be inherited in later generations.

The occurrence of the "inheritance of acquired characters" in any group of organisms is of great interest, and is worthy of careful examination as to its method and significance. Such an examination we undertake in the present chapter. We ask: How does the organic system operate in bringing about genetic variations under the long-continued action of the environmental conditions? How does it happen that a changed environment, although it finally alters the inherited characteristics, does not give an inherited effect until it has acted for many generations? How does it happen that restoration to the original conditions finally causes a return to the earlier characteristics, but not till after many generations?

Something is known as to the nature of the organic system that operates in this way; something is known as to its method of operation. Examination of these matters will compel us to consider some details in the structure and physiology of the organisms; it will illustrate to what lengths nature goes in making difficult a correct interpretation of her activities.

New inherited characteristics acquired under the action of the environment are essentially modifications of the organic

materials on which the characteristics depend. If the acquired characters are indeed inherited, this means that the modified organic materials increase and multiply in their modified condition.

The materials which determine characteristics and their inheritance are, as we know, in the nucleus; they are what we have called the genic materials or genes. Less is known of the genic materials in the Protozoa than in the higher organisms. But it is known that in the Protozoa there are genic materials in the nucleus, and that these control in large measure the characteristics of the individuals. By changing the genic materials of the nucleus, and leaving the rest of the bodily material the same, the inherited characteristics of the entire individual are changed. This has been shown to be true in unicellular organisms, through the processes that occur at conjugation. In conjugation, two individuals unite and ex-change halves of their nuclei (see Figure 18). Just before conjugation, the single nucleus in each individual divides into two halves. Then one-half of the nucleus of individual A passes into B; and one-half of the nucleus of B passes into A. The two halves in each then unite to form a single nucleus. The two individuals then separate.

Thus after conjugation the individual A retains its original cytoplasmic body, and half its original nucleus, but contains in addition a half nucleus from B; and a corresponding change has taken place in B. Now both A and B begin to divide and produce descendants.

Now, the characteristics shown by these descendants prove that the transfer of half a nucleus from B carries with it the power to produce in A the characteristics of B. For after conjugation, the descendants of A are found to be, in the long run, as much like B as they are like A. The half nucleus from B produces a very great change in the characteristics of the
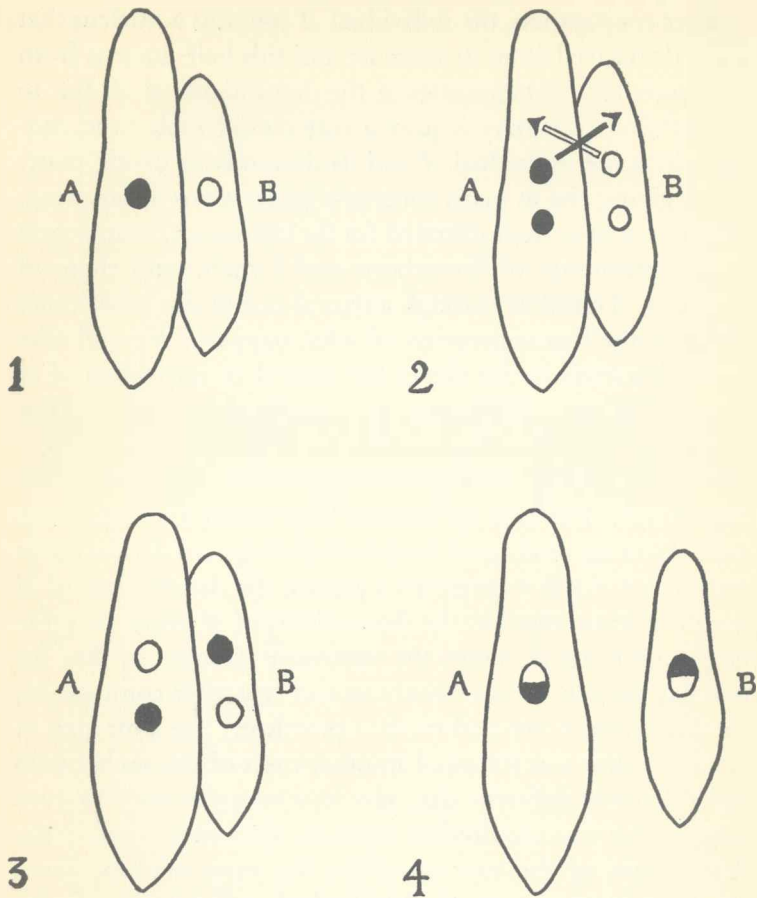
Figure 18. Diagram of the exchange of nuclear materials at conjugation, in Paramecium.

descendants of *A*. It is clear therefore that the nucleus contains materials which affect deeply the development and characteristics of the individual containing them, just as is true in higher organisms.

After conjugation, the individual A contains a nucleus that was half derived from its mate B; and this half nucleus from B changes the characteristics of the descendants of A. But to make this change may require a very considerable time, during which the individual A and its descendants divide many times, giving rise to many successive generations of offspring. All this has been demonstrated for the infusorian Paramecium by the researches of Sonneborn and Lynch,[1] and those of De Garis.[2] To follow through a typical case of size inheritance will give the best conception of what happens. We will take a case described by De Garis. He crossed an individual A of a very large race of Paramecium caudatum, having an average length of 198 microns, with an individual B of a very small race, having an average length of but 73 microns (see Figure 19). The two exchanged half nuclei and separated, each retaining its original length. Each began to divide, about once a day. As the generations passed, the descendants of A began to grow smaller, the descendants of B began to grow larger. Our figure shows the successive changes in size for two-day periods, to the twenty-second day after conjugation. By this time the two had reached practically the same size. A similar course was followed in other cases of crosses between individuals of different size, save that in some cases an even longer time was required to reach the final size, equal in the descendants of the two mates. In the most extreme cases, thirty-six days were required to reach the ultimate size. In the inheritance of fission rates, the new nuclear combination produces its final effect much more quickly. In some cases the fission rate was changed at once after conjugation; within the first five-day period the slow race had begun to divide more rapidly, the fast race more slowly, so that the two were equal. In other cases there is apparently a lag like that which occurs in the inheritance of size, but not lasting so long.
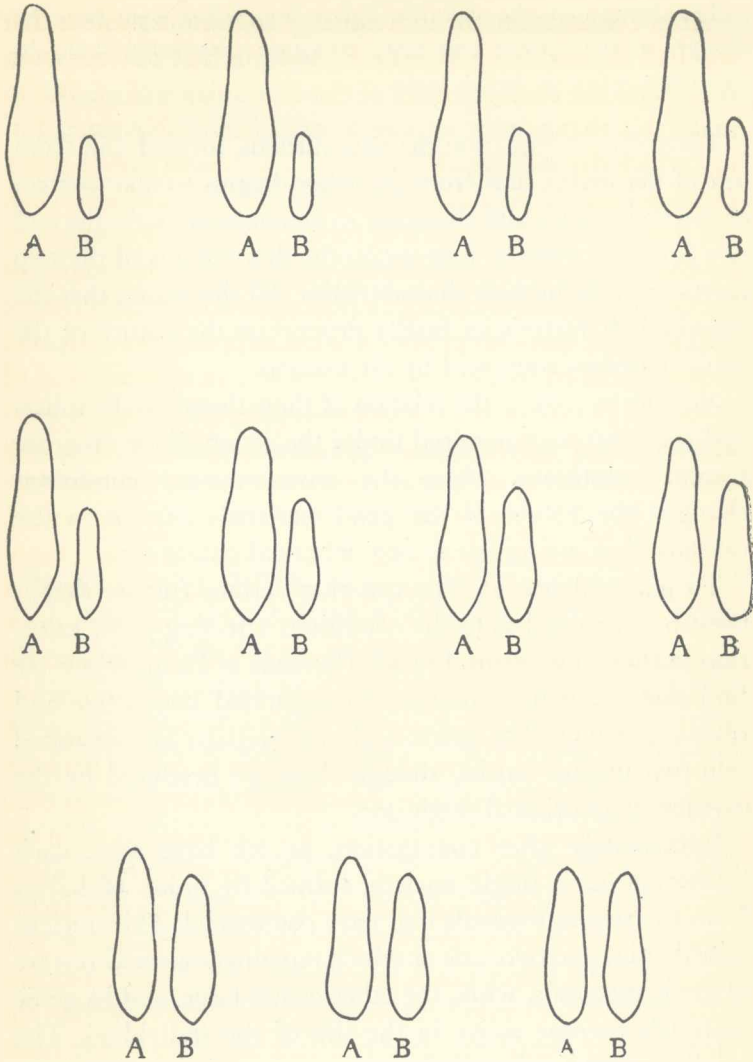
Figure 19. Change in size of the two members of a pair, *A* and *B*, at conjugation, and at two-day intervals after conjugation. At the upper left are shown the relative mean sizes of the two races before

and at conjugation; the successive figures from left to right show the
changes as time passes (see text). Diagram constructed from the
measurements made by De Garis.

Thus after conjugation the new nucleus, formed half from
one of the mates, half from the other, begins to take control
of the body, gradually changing its characteristics. At the end
of a period of three or four weeks the descendants of the two
mates are alike in their characteristics. All this shows that the
inherited characteristics finally depend on the nature of the
genic materials contained by the nucleus.

Now let us look at the relation of these things to the inher-
ited characteristics acquired under the action of the environ-
mental conditions. Have the environmental conditions
changed the nature of the genic materials present in the
nucleus, thus giving rise to new inherited characters?

To get evidence on this question certain further details
must be examined as to the conditions affecting genic mate-
rials in these organisms. In such Protozoa as Paramecium we
find that the genic materials are separated into active and
reserve portions. The active and reserve parts are separated
into two distinct nuclei, though these are produced by the
division of an original single one.

Immediately after conjugation, as we have seen, each
infusorian has a single nucleus, formed by union of halves
from the two individuals that have conjugated. This nucleus
now divides into two, one of which remains as a small reserve
of genic materials, while the other grows large, and its genic
materials become active in the life of the individual. The
small reserve nucleus is known as the micronucleus; the
large active one as the macronucleus. From the macronucleus,
genic materials pass out into the rest of the cell, interacting

with the cytoplasm to produce the structures and functions shown in daily life (Figure 20).

Thus in the infusoria the genic substances are separable into three parts. There is the reserve part in the micronucleus
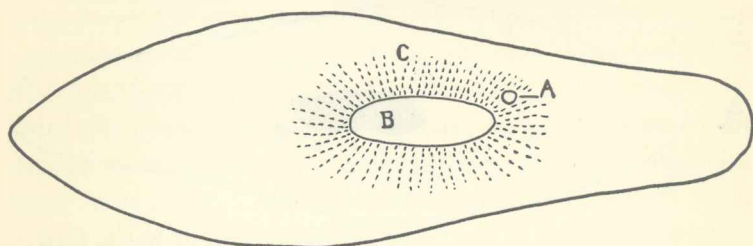

Figure 20. Diagram showing the separation of the genic substances into three parts, in Paramecium. *A*. Micronucleus (reserve). *B*. Macronucleus (active). *C*. Genic materials diffusing into the cytoplasm.

(Figure 20, *A*); there is the active part in the macronucleus (*B*); and there are the much modified parts given off by the macronucleus into the cytoplasm (*C*).

Just before conjugation occurs this large active nucleus, the macronucleus, breaks up and disappears (Figure 21). Pieces of it are seen in the cytoplasm; these gradually become smaller and are absorbed. This absorption of pieces of the nucleus by the cytoplasm presumably affects the nature of the cytoplasm. There is then left but the single micronucleus. This then divides and the two conjugating individuals exchange halves in the way before described.

Look now at the relation between the characters acquired through the action of changed environmental conditions, and these genic materials. The acquired characters are the result of modifications of genic materials. But of what genic materials? Of the reserves, in the micronucleus? Or of the active ones, in the macronucleus? Or of the altered genic products

that have passed out into the cytoplasm and there interact with each other and with other things?

On this point the investigator Jollos, to whom is due so much of our knowledge on the genetics of these organisms,
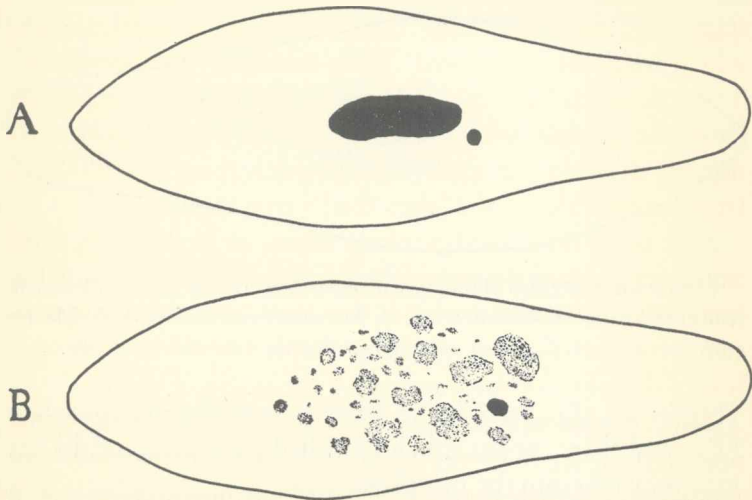

Figure 21. Diagram of the disintegration of the macronucleus in Paramecium. At *A* are seen both micronucleus and macronucleus. At *B*, the macronucleus has broken into pieces, which are being absorbed by the cytoplasm.

has promulgated a widely known theory, according to which none of these environmental modifications affect the reserve genic materials in the micronucleus; they are held to be limited to the genic materials of the macronucleus or of the cytoplasm. For he assumes that any modifications of the genic materials of the micronucleus would be permanent, since the micronucleus is never lost. Since the acquired characters are as a rule not completely permanent, but disappear or are exchanged for others if the animals are kept for a sufficiently

great number of generations in conditions other than those that produced the modifications, Jollos holds that they must have their seat in parts of the cell that are not permanent; that is, in the cytoplasm or the macronucleus. The environmental modifications he holds therefore to be essentially transitory; he calls them therefore *Dauermodifikationen*, which may be translated "long-standing modifications." These he holds "are only changes forced upon the organism from the outside, which do not alter its potentialities, and though they do for some time prevent these potentialities from being realized, the latter finally overcome them."[3]

And since these modifications affect, as he believes, only transitory parts of the organism, leaving the permanent genic materials unchanged, he holds that they "are without significance for the question of the heritable transformation of a line of descent; for the question of the origin of species."

This conclusion rests upon the assumption that the genic materials of the micronucleus must retain permanently any modifications that they undergo. This, however, is not a demonstrated fact. It is certain from the fact of the inheritance of environmental characters that genic materials somewhere in the organism are modified by the environmental conditions. All genic materials come finally from the micronucleus; if they are modifiable in one location they may be modifiable in another. And if they can be modified in one direction by certain conditions, they may well be modifiable in a reverse direction by opposing conditions. The recent discoveries as to inheritance in conjugation taken in connection with the facts as to modification by the environment, certainly suggest an alternative theory to that of Jollos. They suggest strongly that genic materials in the two nuclei may become modified, as well as those elsewhere.

The facts of inheritance at conjugation show with cer-
tainty that the genic materials in both the reserve nucleus and
the active nucleus help to control the characteristics, and they
strongly indicate that the genic products in the cytoplasm play
a part in this also. To see this, follow in the cross between a
Paramecium of a large race and one of a small race what
happens in the descendants of one of the individuals. For
this we may select the small individual *B* in Figure 19, above.
After conjugation it has a small body, composed of cytoplasm
containing genic substances that came from the nucleus of
the small parent *B* before it had conjugated. Its cytoplasm
likewise contains many dissolving pieces of the old macro-
nucleus of the parent *B*. All these parts tend to produce a
body that is small. It also contains a nucleus, half derived
from the large parent *A*, half from *B*. Incidentally, this nucleus
becomes later separated into its two parts, the minute reserve or
micronucleus and the large active macronucleus. This nucleus
tends to make the body large; in the long run it produces a
body of twice the length, and therefore eight times the bulk,
of this original *B*. But during the intervening three weeks,
while at least twenty generations are produced, the body does
not reach this size. It is intermediate between the size that
the new nucleus tends to produce, and the size that the
materials of the old body tend to produce. It is clear therefore
that the materials of the old body help during this period to
control the body size. But as generations pass the new nucleus
more and more dominates the body, until finally a size is
reached which is that due to the new nucleus; a size inter-
mediate between the sizes of the two parents.

During this period of change, the organisms are regularly
dividing; some twenty or more generations are produced
before the final size is reached. At every division the original
material of the body is diluted to half its volume. After ten

generations, any individual contains less than one-thousandth of the original body material; all the rest is new. After fifteen generations the amount of old material remaining in any individual is less than one thirty-two-thousandth of the original body. Yet at these times there is still a marked distinctive effect of the original body material; the size is not yet that which the nucleus tends to produce. Clearly, either this original body material has a great effect even in enormously high dilutions; or what seems rather the case, some part of it has the power of reproducing itself true to type during division, just as does the genic material of the nucleus, so that we must call it also genic material. This genic material of the body is either the cytoplasm itself, or some remnants of the old macronucleus. But this original body material gradually loses its distinctive effect, through coming under the influence of the nucleus. After three or four weeks or twenty to thirty generations, it is completely dominated by the new nucleus.

With these facts in mind, return to our question: Are the inherited environmental characters the result of modification of the reserve genic materials of the micronucleus, of the active genic materials of the macronucleus, or of the genic products present in the cytoplasmic body?

If the environmental modifications are in the genic materials of the cytoplasm only, we should expect the acquired characters to disappear in twenty to thirty days, since we know from what happens at conjugation that by the end of such a period the nucleus has taken full control. Again, if the acquired characters are in the active macronucleus only, they would disappear at once when the macronucleus disappears. If they are due to modifications of the micronucleus, they would disappear only when some new influence modifies this micronucleus.

It is clear that the acquired characters do not disappear in any such brief time as thirty days; on the contrary they may persist for many months. This appears to be strong evidence against the limitation of the environmental effect to the cytoplasmic genic materials.

At certain times in the course of generations, as we have seen, the large macronucleus disappears; it breaks into pieces, and these are absorbed, though they may remain for a few days in the cytoplasm. This leaves the micronucleus as the only nucleus. If the modifications resulting from the action of the environment are limited to the active genic substances of the macronucleus, they should disappear when the macronucleus disappears; or at least a little later, after its pieces in the cytoplasm have disappeared. At this time the acquired characters should be lost.

Sometimes the acquired characters are indeed lost when the macronucleus is lost. At the time of conjugation the macronucleus is lost in both individuals, and is replaced from the new nucleus formed from the union of the two half nuclei —one half from each of the individuals of the pair.

And at conjugation certain of the inherited modifications partly or entirely disappear. This is true to a large extent of the injurious modifications resulting from bad living conditions. They often almost or quite entirely disappear when conjugation occurs, so that conjugation has been said to cause "rejuvenescence." The animal after conjugation regains its original high level of vitality, even though it remains in the conditions which brought about the injuries. It is also true that the increased resistance produced by long subjection to certain chemicals, or by heat, often disappears at conjugation. Since the macronucleus disappears at the same time, this could be held to indicate that it is the genic substances of the macronucleus that have been modified, while those of the

micronucleus were unaffected. When the macronucleus disappeared, therefore, the acquired modifications also disappeared.

But disappearance of the macronucleus and its replacement from the micronucleus are not all that occurs at conjugation. There is also the exchange of half nuclei; so that each individual has a new combination of genic materials. This might well give a new combination of characteristics, so that the old characteristics, however acquired, might be no longer manifested. The seeming loss of the acquired characters might be due to this formation of a new combination of genic materials, rather than to the loss of the macronucleus.

The difficulty here presented was overcome in the infusorian Uroleptus by an experiment of Calkins. In a conjugating pair from a depressed stock, by an operation Calkins prevented the exchange of the half nuclei. The macronucleus disappeared as usual, but the new one was formed from the individual's own micronucleus, without aid from that of its mate; so that there was no new combination of genic materials. Yet the descendants of the individual in which this occurred had lost their depression. With the disappearance of the macronucleus the acquired modification likewise disappeared.

The same thing is shown to be true for the infusorian Didinium by Calkins and by Beers. When the animals become depressed through the action of bad conditions, they become encysted, and while in the cyst the macronucleus seemingly disappears and is replaced from the micronucleus. At the same time the depressed condition of the organism also disappears.

In these cases then it appears probable that the modification produced by the environment was only in the genic materials of the macronucleus. The macronucleus is a transi-

tory body; it disappears at intervals, and when it disappears, the acquired characteristics dependent on it must disappear also. Such acquired characters then are only transitory. They play an important rôle in the life of the organisms; they last for generations, but they do not permanently alter the characteristics of the species. For such modifications Jollos' term of *Dauermodifikationen* is an appropriate term; they are longstanding modifications, but destined to disappear when the macronucleus disappears.

But it is certain that not all environmental modifications are limited to the macronucleus. In Paramecium, the organism most studied with respect to these matters, it was unexpectedly found, after much work had been done, that the macronucleus disappears at regular rather short intervals, being replaced in each case from the micronucleus. This is the process known as endomixis. In Paramecium aurelia this disappearance and replacement occurs every three or four weeks; in Paramecium caudatum the interval is about twice as great.

Yet most of the acquired modifications do not disappear at these periods. Though the macronucleus is lost, they remain. It is clear therefore that they are not modifications limited to the genic materials of the macronucleus. This is true of the depression, or injurious modifications, induced by poor living conditions. Paramecium continues to "run down," becoming more and more depressed, in spite of repeated replacements of the macronucleus from the micronucleus. This is true also in many cases of the inherited resistance induced by subjection to heat or to chemicals; this often does not disappear when the macronucleus disappears. On the contrary, it continues to be inherited for many generations, during which there are repeated disappearances and replacements of the macronucleus.

All this shows that other genic materials besides those in the macronucleus may become modified by environmental conditions. We have already given reasons for holding that the cytoplasmic genic substances cannot be the seat of the environmental modifications; for if they were, the modifications would be expected to disappear inside of about four or five weeks. There remains only the genic materials of the micronucleus itself. These reserve genic materials would doubtless be modified less readily than the active materials in the macronucleus; but there is no reason to reject absolutely the possibility of modification for them, for they are literally genic materials of the same type as those in the macronucleus, since the two are halves of the same original nucleus.

Furthermore, in certain cases a second-degree modification is induced, of such a character as to suggest strongly that its seat is in the micronucleus. In Uroleptus and in Spathidium it has been shown that when the depression induced by bad conditions is moderate in degree, it is completely lost at conjugation when the macronucleus is replaced. But if the depression has become very severe, it does not fully disappear at conjugation, even though the macronucleus is then removed. The condition of the animals is improved, but a considerable degree of depression remains and continues to be inherited. This is what we should expect if the environmental conditions first modify the genic materials of the macronucleus, then if severe and long continued, modify the reserves in the micronucleus.

The facts therefore appear to point to the following situation. Environmental conditions, so far as they induce inherited modifications, first alter the active genic materials of the cytoplasm; such modifications last but a short time. Later, after acting longer, they alter the genic materials of the macronucleus; such modifications disappear with the disap-

pearance of the macronucleus. Finally, after severe and long-continued action, the environmental conditions alter the reserve genic materials in the micronucleus. Such modifications last either until new environmental conditions again alter the micronucleus; or until through conjugation a new combination of genic substances is made that yields new characteristics.

A final test of whether these intense modifications do indeed affect the micronucleus must come through crossing a deeply modified stock with one that is not modified, thus determining whether the modification is transmitted with the micronucleus. By the methods recently devised by Sonneborn and by De Garis, such a test may come soon.

We may attempt now a summary of the established facts as to the inheritance of acquired environmental characters in unicellular animals. Some of the inherited characteristics manifested by these organisms are consequences of the type of environment under which their ancestors have lived; in some cases ancestors hundreds of generations earlier. A changed environment induces a change in the genic materials that persists long after removal from the conditions that induced the change. And this altered genic material, as it assimilates, grows and multiplies, produces, not the original type of material, but the modified type. This continues for many generations.

Neither the original characteristics nor the modified characteristics are fully stable. The original characteristics are found to become modified as the conditions alter. In this respect they are like the modified characters; these too become altered again when the original conditions are restored. There is then a return to the original characteristics, and this return is to be expected on the same ground that the modifi-

cations were to be expected. One set of conditions produces (if it acts for a sufficiently long period) the original characteristics; another set (by long-continued action) produces the modified characters. The striking feature of these changes in characteristics is that they lag far behind the conditions that finally induce them. When the organisms are cultivated in such a chemical as arsenic, it may be many generations before their resistance to this chemical is increased; when they are returned to "normal" conditions, it may again be many generations before the "normal" resistance is again produced. In either case, the characteristics shown by the organism depend largely on the conditions in which its ancestors of long past generations have lived.

How are to be explained the adaptive features of the genetic variations induced by environment? How is to be explained the acquirement, under the action of particular agents, of inherited acclimatization or immunity; increased powers of resistance to these agents? To attribute such results to a general quality of adaptiveness does help, for in each example of adaptation the result is always a specific relation between a particular organism and a particular situation or condition; and the question for answer is: How is this specific relation brought about? How is to be explained the acquirement, under the action of a particular chemical, of increased powers of resistance to that particular chemical? When the organisms are placed in contact with nutritive material which they cannot use, how is to be explained the acquirement of a changed method of metabolism, enabling them to use precisely that material?

These questions are identical with those that arise with relation to the powers of direct acclimatization or immunization that are found in the somatic tissues of higher organisms. There is as yet no assured answer to the questions. In an

earlier work[4] the present author has developed the view that such adaptive action must be due fundamentally to selective action occurring within the tissues of the organism. If there is such action, it occurs as well in the genic materials of unicellular organisms as in the somatic tissues of higher forms.

Does the fact that environmental effects and reactions are inherited in Protozoa, giving rise to adaptive and progressive inherited characteristics, suggest that we should eventually expect to find this method of operation in the higher organisms?

Perhaps the most important feature in the genetics of the Protozoa is the demonstration that the genic materials possess here the same powers of direct acclimatization or immunization to injurious agents that are possessed by the somatic tissues of higher organisms. Genic materials are materials that assimilate and reproduce, each true to its type. The facts in the Protozoa show that after the genic materials have undergone an adaptive change, they may assimilate and reproduce in the changed condition, resulting in inheritance of the change. There is thus no incompatibility between the acquirement of adaptive changes and the capability of assimilation and reproduction in the changed condition; for these things occur in the same material in lower organisms. There is no inherent impossibility in this method of operation. There is thus no general reason why it should not occur in higher organisms; no a priori reason why the germ cells of higher animals should not thus acquire certain adaptive characteristics, and hand them on to descendants. Whether they do or do not is simply a question of fact, to be determined by observation. If the higher organisms differ from the lower ones in this matter, it is because of special conditions operating in higher organisms.

There are indeed such special conditions working against the inheritance of environmental effects in multicellular organisms. As compared with unicellular organisms, the differences that are significant in this respect are mainly the following. The unicellular organisms are minute, their genic materials are in close relation with environmental agents. The higher organisms are relatively enormous. Only the genic materials of their germ cells can affect the characteristics of descendants, and the germ cells are hidden deeply within the body, protected from the action of most outer agents. Between parent and offspring a long series of cell generations intervenes, during which there is opportunity for loss of any modifications induced in the germ cells of the parent. These conditions operate against the inheritance of the effects of environmental agents in higher organisms.

Weighing more strongly than any of these considerations perhaps is the fact of the great weight of negative evidence from observations and experiments directed toward testing the inheritance of environmental acquirements. No subject in biology has been worked on more than this. The overwhelming majority of the results are negative. Sometimes the discovery of the inheritance of acquired characters is announced; usually it is discovered later that an error underlies the interpretation. As yet outstanding are the results of William McDougall[5] on the inheritance of the effects of training on the rat. These if accepted at face value would demonstrate the inheritance of acquired characters. In view of the history of many such investigations in the past, one can hardly do otherwise than expect that some error of interpretation will be found to underlie these results; and that they will be found not to demonstrate the inheritance of the effects of training. It may turn out that the inheritance of environmental adaptations is limited to unicellular organisms.

If this is the situation, where are we left as to the source of the variations in genic materials that must underlie evolution in higher organisms?

Some investigators have expressed the opinion that such variation is not required; that mere change of combination and grouping, without alteration in the nature of any gene, can in some way give rise to progressive evolution; can have produced the seeming differentiation among genes now existing. To most, however, this appears unintelligible.

Possibly a type of gene change that is in the nature of development rather than in that of disintegration and abnormality will eventually be detected. When this is demonstrated, the road for progressive evolution will be open; until this is demonstrated genetic science appears to be left with the task that Osborn has called The Search for the Unknown Factor in Evolution.
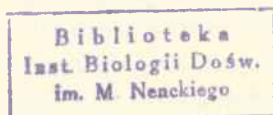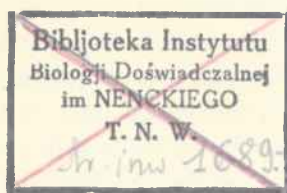
NOTES ON CHAPTER V

[1] Page 122. T. M. Sonneborn and R. S. Lynch, "Hybridization and Segregation in Paramecium aurelia," *Journal of Experimental Zoology*, 1934, Vol. 67, pp. 1-72.

[2] Page 122. C. F. De Garis, "Heritable Effects of Conjugation between Free Individuals and Double Monsters in Diverse Races of Paramecium caudatum." To appear in the *Journal of Experimental Zoology*.

[3] Page 127. V. Jollos, "Experimentelle Vererbungsversuchen an Infusorien," *Zeitschrift für induktive Abstammungslehre*, 1920, Vol. 25, p. 89. See discussion of this matter in Jennings, *Genetics of the Protozoa*, p. 299.

[4] Page 136. H. S. Jennings, *Behavior of the Lower Organisms* (New York, 1906), Chapter XXI.

[5] Page 137. See J. B. Rhine and William McDougall, "Third Report on a Lamarckian Experiment," *British Journal of Psychology*, Vol. 24, 1933, pp. 213-24.