

## CHAPTER III

The Basis of  
Individual Variation

IN THE PREVIOUS chapters we have studied variation on two different levels; first, that between different individuals of a population and, second, the statistical variation between populations and population systems. Although the variation which is responsible for the origin and divergence of evolutionary lines is entirely on the population level, this variation is itself a product of differences between individuals. Heritable individual variations are the basic materials of evolution; the forces acting on populations are the mechanisms which fashion these materials into an orderly, integrated pattern of variation. Obviously, therefore, our understanding of evolution must come from an understanding of differences both between individuals and between populations.

## ENVIRONMENTAL MODIFICATION AND ITS EFFECTS

The variation seen between the individuals of any population is based on three factors: environmental modification, genetic recombination, and mutation. Of these, environmental modification is the least important in evolution, although sometimes very conspicuous. As mentioned in Chapter I, the first step in any analysis of natural variation is the performing of transplant experiments by which the effects of environmental differences are largely neutralized. Such experiments have shown that each genotype has its own genetically determined degree of modifiability or plasticity (see Fig. 3). The adult individual we see, therefore, is a phenotype which is always the product of the effect of a given environment on an individual with a particular hereditary background, or genotype. That this axiom of genetics disposes at once of many futile arguments as to the relative importance of heredity and environment or "nature and nurture"

has been pointed out by many geneticists, including Dobzhansky (1941, pp. 16-17).

Recent transplant experiments, as exemplified by the work of Clausen, Keck, and Hiesey (1940), have shown that the phenotype can be altered much more profoundly in some characteristics than in others. Most easily modified is the absolute size of the plant and of its separate vegetative parts, that is, roots, stems, or leaves. Hardly less plastic are the amount of elongation of the stem, the number of branches, and the number of leaves, inflorescences, or flowers. The quantity of hairiness or pubescence also is relatively easily modified. On the other hand, many particular characteristics of individual form and pattern can be modified only slightly or not at all by the environment; their appearance in the phenotype is almost entirely the expression of the genotype. Such constant characteristics are in *Potentilla* the pinnate character of the leaves; the type of serration of the leaf margins; the type of pubescence; whether glandular or nonglandular; the shape of the inflorescence; and most of the floral characteristics, such as the size and shape of the sepals, the petals, and the carpels. This relative plasticity of certain vegetative characteristics and constancy of reproductive ones has long been realized by plant systematists; upon it is based the greater emphasis in classification on reproductive characteristics as compared to vegetative characteristics.

The basis of this differential plasticity is probably to be found in the manner of growth of the plant shoot. The easily modified characteristics such as absolute size, elongation, branching, and number of parts are determined by the length of time during which the shoot meristems are actively growing and on the amount of cell elongation which takes place during the later expansion of the various organs of the plant. These processes are relatively easily affected by such external factors as nutrition, water supply, light, and temperature. On the other hand, the fundamental morphological pattern of individual organs, such as bud scales, leaves, and the parts of the flower, is already impressed on their primordia at a very early stage of development (Goebel 1928, 1933, Foster 1935, 1936) when the influence of the external environment is at a minimum. The relative stability of the reproductive structures is due largely to the fact that they are

differentiated, not in a simple serial fashion as are the leaves and the branches, but more or less simultaneously or according to a rather complex development pattern (see Chapter XIII). Furthermore, the growth of the primordia after their differentiation is usually less in floral parts than it is in leaves and branches.

Many aquatic or semiaquatic plants are an exception to the usual rule that the shape pattern of organs is little modified by the environment. The difference in outline and internal structure between the submersed and the aerial leaves of such plants as *Myriophyllum verticillatum* has long been used as an example of the extreme modification of phenotypic expression by the environment. Other aquatic types, such as *Sium*, *Polygonum amphibium*, and various species of *Sagittaria* and *Potamogeton*, are equally capable of modification in their leaves (Fernald 1934). The difference between an aqueous medium and an aerial medium is one of the most effective external forces in modifying the organization and growth of the vegetative meristem and its appendages. In all these aquatic species, however, the reproductive parts are constant, no matter what the nature of the external medium.

In this discussion of the relative plasticity of vegetative as compared to reproductive characteristics, emphasis must be placed on the fact that we are dealing only with the modifiability or plasticity of the individual genotype and phenotype. The term "plasticity" is often used to denote variability between genotypes of a population or species. This, of course, is a totally different phenomenon and, as will be pointed out later in this chapter, is governed by entirely different causes. There is no a priori reason for assuming that genotypic variation between individuals should be greater in vegetative characteristics than in reproductive characteristics. In fact, there are many plant species, particularly among those of garden flowers, in which the variation between individuals and varieties is much greater in the reproductive parts of the plant than it is in the vegetative parts.

Environmental modification is a source of variability which must be kept in mind by the evolutionist because it affects every individual we see in nature. But as a direct factor in evolutionary divergence it is not significant. The Lamarckian concept of evolution through the direct effect of the environment on the individual, and the inheritance of acquired modifications, has in the past

seemed even more attractive to explain evolution in the plant kingdom than in animals. As has just been mentioned, the genotypes of plants are relatively easily modified by environmental agencies, such as temperature, moisture, and nutrition, which are simple and ever present. Furthermore, the type of modification induced by the environment frequently simulates that which is produced by genetic factors. For instance, many species of plants possess genotypes which normally promote erect growth, but produce prostrate and spreading phenotypes when the plants are exposed to the extreme conditions of wind and salt spray prevailing at the seacoast. In certain of these species, such as *Succisa pratensis*, *Atriplex* spp. and *Matricaria inodora*, maritime ecotypes exist in which the prostrate spreading character is determined genetically and is maintained even in garden cultures (Turesson 1922b). Furthermore, the principle established by Weismann of the separation between somatoplasm and germ plasm does not hold for plants as it does for animals. In any perennial plant, the apical meristem produces alternately vegetative and reproductive organs, and any permanent modification of these meristematic cells at any time will affect the gametes they will eventually produce, and thereby the individual's progeny. Theoretically, therefore, the direct alteration of heredity in adaptive response to a changed environment would seem on the surface not impossible.

But the actual facts are that in plants, as in animals, there is no valid experimental evidence to indicate that acquired characters are inherited, and some experiments exist which show that they are not. The extensive transplant experiments of Clausen, Keck, and Hiesey (1940), already discussed many times, have failed to give any evidence of this type of inheritance. An earlier experiment which has the advantage of including a relatively large number of generations is that of Christie and Gran (1926) on cultivated oats.

This does not mean that the hereditary material cannot be altered by the environment. On the contrary, the effect on the germ plasm of such environmental agencies as heat, cold, radiation, and certain chemical substances is well known. But at least in the higher organisms the changes induced by extreme environmental conditions are either at random, like the ap-

parently spontaneous mutations of the germ plasm, or of a specific nature, like the induction of polyploidy by means of heat shocks or the action of colchicine. In any case, they do not cause the progeny of the organism to become better adapted to the environmental agency used.

#### THE IMPORTANCE OF RECOMBINATION

The second factor in variation, recombination, is the main immediate source of variability for the action of selection and other external forces which direct the evolution of populations. By far the greater part of the genotypic variation in any cross-breeding population is due to the segregation and recombination of genic differences which have existed in it for many generations. This fact is brought into striking relief when one raises under uniform conditions the progeny of any single plant of a self-fertilized species. Such progenies are remarkably uniform, in striking contrast to the variable progeny obtained from a single plant of any cross-pollinated species. New mutations are undoubtedly occurring constantly in most natural populations, but they are so rare that their total effect on genotypic variability in the generation in which they occur is negligible. As Sumner (1942) has pointed out, mutations become material for alteration of the population by selection only after they have existed in it for several generations, and the primary adaptation to any new environment is accomplished by segregating variants of a heterozygous population.

The importance of sexual reproduction and cross-fertilization stems directly from this fact. For this reason, a particular study of the action of different types of sexual reproduction is essential to an understanding of the dynamics of evolution. Since such a study must be made on the basis of the importance for efficient natural selection of each type of reproduction, it will be deferred to Chapter V, following the discussion of selection.

#### TYPES OF MUTATION AND THEIR SIGNIFICANCE

Although the immediate basis of the variation which makes evolution possible is genetic segregation and recombination, its ultimate source is mutation. This fact is implicit in the definition of mutation, as accepted by most modern geneticists. Good dis-

cussions of the history and meaning of this term have been given by Dobzhansky (1941, pp. 19-22) and Mayr (1942, pp. 65-70); they need not be repeated here. A brief and satisfactory definition is given by Mayr (p. 66): "A mutation is a discontinuous chromosomal change with a genetic effect." One should understand clearly, though, that the adjective "chromosomal" refers to chemical changes in a small part of the chromosome, as well as to alterations of its physical structure. A particularly significant fact about this definition is that the chromosomal change rather than the genetic effect is characterized as discontinuous. This emphasizes the presence of mutations having less visible effect on the phenotype than the changes produced by environmental modification or by the genetic effect of segregation and recombination in a typical heterozygous natural population. Such mutations with slight effect, since they do not produce a visible discontinuity in any character, blend into the continuous or fluctuating individual variation normally present in panmictic populations. They contrast sharply with the type of mutations originally described by De Vries, which produced striking and discontinuous variations in the phenotype.

Mutations may be classified either on the basis of the changes taking place in the chromosomes or according to their effect on the phenotype. The former classification is the one most widely used. In it we may recognize four main groups: first, multiplication of the entire chromosome set, or polyploidy; second, the addition or subtraction of one or a few chromosomes of a set; third, gross structural changes of the chromosomes; and fourth, submicroscopic changes, probably including chemical alterations of the chromosomal material. The first of these, polyploidy, is of such special significance that it will be treated in detail in Chapters VIII and IX. The second includes chiefly polysomic or monosomic types which possess an extra whole chromosome or are deficient for one. Such types are partly sterile and genetically unstable, and therefore of relatively little significance in evolution. They are very rarely found in nature. Other types of aneuploid changes in chromosome number occur, but they are usually associated with rearrangements of chromosomal segments. They are discussed in Chapter XII.

The third type of mutation, gross structural alteration of the

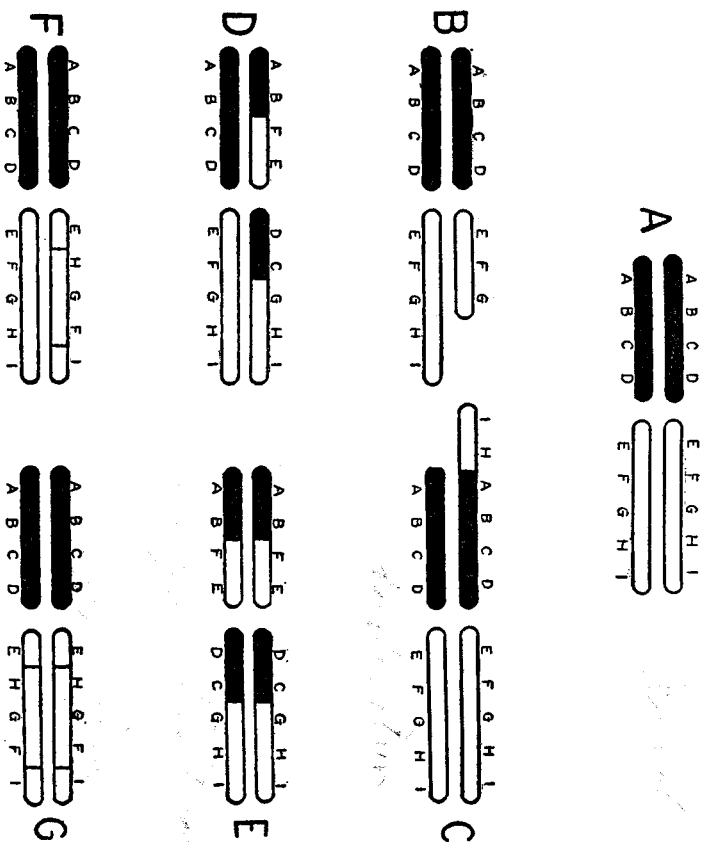


Fig. 8. (a) Normal chromosomes; (b) deficiency; (c) duplication; (d) heterozygous translocation; (e) homozygous translocation; (f) heterozygous inversion; (g) homozygous inversion. From Dobzhansky 1941.

chromosomes, is of considerably greater importance. These changes are discussed in detail by Dobzhansky (1941, Chapter IV), to whose book, as well as to any modern book on cytology, the reader is referred. The four principal types of such changes are deficiencies, duplications, translocations, and inversions. These are diagrammatically shown in Figs. 8-10, taken from Dobzhansky (1941).

Deficiencies are usually lethal or semilethal when homozygous, so that the majority of them are of relatively little importance in evolution. It is possible, however, that a considerable proportion of the genetic changes which have been regarded as point mutations are actually minute deficiencies (McClintock 1938, 1941, 1944). Within the limits of four chromosomes in the short arm of chromosome 5 of maize, McClintock (1941) found six distinct nonallelic mutations. All of these appear to be homozygous minute deficiencies. A similar series was later (1944) produced in the

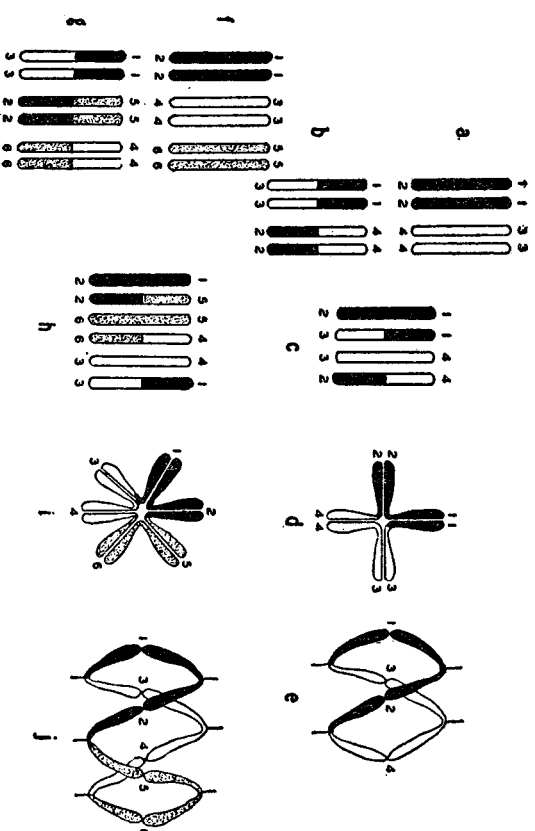


Fig. 9. Translocation between two (b-e) and between three (g-i) chromosomes. Normal chromosomes (a) and (f); (b) and (g) translocation heterozygotes; (c) and (h) translocation homozygotes; (d) and (i) chromosome arrangement at pairing stages; (e) and (j) arrangement of chromosomes at the metaphase of the meiotic division. From Dobzhansky 1941.

short arm of chromosome 9. There is no reason to believe that these regions are more mutable than other parts of the maize complement or that the maize plant is unusual in the type of mutations it can produce. Similar minute deficiencies simulating mutations have been produced by several workers in *Drosophila*. In some groups of organisms, therefore, minute deficiencies may play a role in evolution similar to that of point mutations.

Duplications may be of even greater significance than deficiencies, although as yet not much is known about them. They are most easily detected in the giant chromosomes of the salivary glands of *Drosophila*, *Sciara*, and other flies, where they appear as "repeats" of certain patterns of arrangement of the dark staining bands. In plants, their spontaneous occurrence can be inferred chiefly from the behavior of chromosomes at meiosis in haploids or in species hybrids of certain types. Thus, in haploid *Oenothera* (Emerson 1929, Catchside 1932), barley (*Hordeum distichum*, Tometorp 1939), rye (*Secale cereale*, Levan 1942a), *Triticum monococcum* (L. Smith 1946), and *Godetia whitneyi*

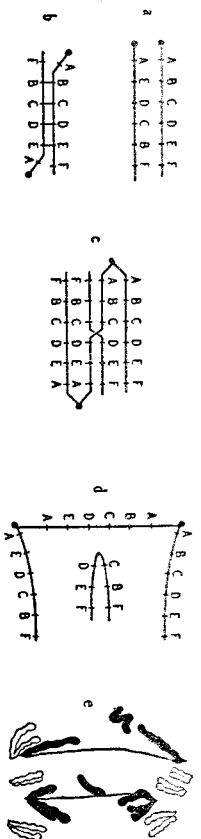


Fig. 10. Crossing over in inversion heterozygotes, leading to the formation of chromosomes with two and with no spindle attachments (d). (e) Chromatin bridges in the hybrid *Lilium martagon*  $\times$  *L. hanseini*. (After Richardson.) From Dobzhansky 1941.

(Håkansson 1940), there is occasional pairing of chromosomal segments. Although some of this pairing may be the association of nonhomologous, genetically different segments, as suggested by Levan (1942a), nevertheless the work of McClintock (1933) on *Levan* (1942a), nevertheless the work of McClintock (1933) on nonhomologous association in maize suggests that this phenomenon does not occur extensively unless the pairing chromosomes have some homologous segments in common. If these chromosomes are different members of a single haploid set, then this set must contain duplications. In the hybrid between *Bromus calharrticus* ( $n = 21$ ) and *B. carinatus* ( $n = 28$ ) the normal pairing at meiosis is 21 pairs and 7 single chromosomes (Stebbins and Tobgy 1944). But in a very few cells two of these univalents will pair with each other in a single small segment, indicating the presence of a duplication.

Dobzhansky (1941, p. 129) and, particularly, Metz (1947) have emphasized the importance of repeats or duplications as the only method, aside from polyploidy, by which the number of genes in the germ plasm of an organism may be increased. Just how essential to evolutionary progress is an increase in the number of genes cannot be decided until more is known about the physico-chemical structure of chromosomes and genes and about the nature of mutations. It must be mentioned here, however, that aside from polyploidy there is no evidence of a regular increase in the amount of chromosomal material during the evolution of the land plants. The most archaic types known, the Psilotales and the Ophioglossales, have larger chromosomes than the majority of flowering plants. In the larger subdivision of the angiosperms, the dicotyledons, most of the species with the largest chromosomes are in the most primitive order, the Ranales (*Paeonia*, *Helle-*

*borus*, *Delphinium*, *Ranunculus*, *Podophyllum*), while the most highly evolved types (Labiales, Scrophulariaceae, Compositae, and so on) usually have small or medium-sized chromosomes. Since the genes themselves probably constitute only a small fraction of the substance which makes up the chromosomes, we cannot say whether or not differences in the size of the chromosomes are correlated with differences in the number or size of the genes which they contain. Nevertheless, plant cytology provides no evidence in favor of the hypothesis that progressive evolution has been accompanied by a regular increase in the number of gene loci contained on each chromosome. A more likely hypothesis would be that progressive physicochemical diversification of a more or less constant amount of chromosomal substance is responsible for increasing evolutionary specialization. The role of duplications or repeats might be to act as "buffers," offsetting certain deleterious effects of otherwise beneficial mutations.

The two remaining types of gross structural changes, translocations and inversions, are much better known. Translocations are common in a number of plant species, where they are recognized by the presence of rings or chains of chromosomes at meiosis in individuals heterozygous for them (Fig. 9). Typical examples are *Datura stramonium* (Blakeslee, Bergner, and Avery 1937), *Campanula persicifolia* (Gairdner and Darlington 1931, Darlington and Gairdner 1937), *Polemonium reptans* (Clausen 1931a), species of *Tradescantia* (Darlington 1929a, Sax and Anderson 1933, Anderson and Sax 1936), and *Triticum monococcum* (Smith 1936). Further examples are cited by Darlington (1937) and Dobzhansky (1941, pp. 114-115). The accumulation of translocations to give structural heterozygotes with most or all of their chromosomes associated in a system of rings or chains, as in *Oenothera*, *Rhoeo*, and the North American species of *Paeonia*, is a special phenomenon which will be discussed in Chapter XI.

Inversions of chromosome segments are likewise well known and probably occur in an even larger number of plant species than do translocations. They can readily be detected in the heterozygous condition in species like maize, in which chromosome pairing at mid-prophase (pachytene) of meiosis is easily observed by the presence of a loop, formed by the pairing of the normal with the inverted chromosome segment (McClintock

1933; see diagram, Fig. 7, of Dobzhansky 1941). In most species of plants, however, this stage cannot be easily observed, and inversions are recognized only by the results of chiasma formation and crossing over in the inverted segment. This leads to the formation at meiotic metaphase and anaphase of one chromatid with two spindle fiber attachments and one free, acentric fragment (Fig. 10; see also Darlington 1937, pp. 265-271). Since the fragment always includes the entire inverted segment plus those parts of the exchange chromatids distal to it, the size of the fragment indicates the position (not the size) of the inversion on the chromosome arm. The frequency of bridge-fragment configurations in any species or hybrid obviously depends on three factors: chiasma frequency, number of inversions, and size of inversions.

A considerable list of plant species containing individuals heterozygous for inversions is given by Darlington (1937, pp. 273-274), as well as by Dobzhansky (1941, p. 126). These lists could be greatly extended on the basis of more recent observations, and interspecific hybrids are even more likely to be heterozygous for inversions. The two genera which up to date seem to exceed all others in the occurrence of detectable inversions are *Paris* and *Paeonia*. Geitler (1937, 1938) found that in Tirolean populations of *Paris quadrifolia* every individual is heterozygous for one or more inversions, which are distributed over every chromosome arm in the entire complement. Stebbins (1938a) found a greater or lesser percentage of cells with bridge-fragment configurations in every one of the Old World species of *Paeonia* investigated by him, and this is likewise true of every individual of the two New World species, *P. brownii* and *P. californica* (Stebbins and Ellerton 1939, Walters 1942). In one species, *P. triternata* var. *mlokosewitschii*, 20 percent of the cells contained these configurations. Although the different chromosome arms could not all be distinguished from each other, in all probability the inversions in this species, as in *Paris quadrifolia*, are scattered through the entire chromosomal complement. Analysis of the different bridge-fragment configurations found in a single chromosome arm of an interspecific hybrid, *Paeonia delavayi* var. *lutea*  $\times$  *P. suffruticosa*, showed that this arm was heterozygous for several inversions, probably of small size. In all likelihood, therefore, the high frequency of bridge-fragment configurations in species of *Paeonia*,

and perhaps also in *Paris*, is due to the presence of many small inversions rather than a few large ones. If this is true, then the actual frequency of inversions in many other cross-fertilized plant species may approach or equal that in *Paris* and *Paeonia*. These two genera have among the largest chromosomes in the plant kingdom, so that inverted segments which are relatively short in relation to the length of the chromosome would nevertheless be long when compared to the total length of the chromosomes in other species. It is possible, therefore, that in species with smaller chromosomes inversions as small in proportion to the total length of a chromosome arm as are many of those in *Paris* and *Paeonia* would never form chiasmata when paired with a normal segment. The fact must be emphasized that the evidence from bridge-fragment configurations indicates only the minimum number of inversions for which an organism is heterozygous, and may come far from revealing all of them.

The evidence presented above suggests that translocations and inversions occur frequently in most species of plants. They do not, however, produce any recognizable effect on either the external morphology or the physiological adaptive characteristics of the species. Examination of numerous translocation types produced artificially in maize (Anderson 1935), *Crepis* (Levitzy 1940, Gerasimova 1939), and other plants has failed to show any conspicuous morphological effect of these chromosomal alterations. The same is true of gross structural differences in the chromosomes of wild species. Blakeslee (1929) and Bergner, Satina, and Blakeslee (1933) found that the "prime types" of *Datura*, which differ from each other by single translocations, are morphologically indistinguishable, while differences between individuals are no greater in those species of *Paeonia* which are heterozygous for a large number of inversions than they are in those with relatively few inversions (Stebbins 1938). In *Paeonia californica*, furthermore, some populations are structurally homozygous or nearly so and others contain a great array of different interchangeable heterozygotes (Walters 1942 and unpublished). These two types of populations are indistinguishable in external morphology in respect to both individual variation and the differences between populations. In plants, the phenomenon of position effect, which has considerable importance in *Drosophila*, has

been clearly demonstrated only in *Oenothera* (Catchside 1939, 1947a). It appears to be so uncommon that it has relatively little importance in evolution. Gross structural alterations of the chromosomes are not the materials that selection uses to fashion the diverse kinds of organisms which are the products of evolution.

The importance of these chromosomal changes, however, lies in an entirely different direction. In most structural heterozygotes there is a certain amount of chromosomal abnormality at meiosis, leading to the production of a certain percentage of inviable gametes. This is relatively small when single translocations or inversions are involved, so that individual structural differences scattered through the chromosome complement cause a relatively slight reduction of fertility. But when individuals differ by groups or complexes of inversions and translocations, particularly if these are closely associated in the same chromosome arm, pairing at meiosis may be considerably disturbed, and even with relatively normal pairing the effects of crossing over and chromosomal segregation will be the production of gametes containing duplications and deficiencies, and therefore inviable. Gross structural changes of the chromosomes, therefore, are the units from which are built up many of the isolating mechanisms separating plant species. These mechanisms will be discussed in greater detail in Chapter VI, in connection with species formation.

From the summary presented above we may conclude that the majority of the morphological and physiological differences important in evolution come about, not through alterations of the number and gross structure of the chromosomes, but through changes on a submicroscopic level; the "point mutations" of classical genetics. This conclusion agrees in general with that of most geneticists, such as Sturtevant and Beadle (1939), and was also reached by Babcock (1942, 1947) as a result of his prolonged and intensive studies of cyto-genetics and evolution in the genus *Crepis*. The physicochemical nature of these mutations is not known. That they represent a class of phenomena different from larger structural changes in the chromosomes is suggested by the results of Stadler and Sprague (1936) on the effects of ultraviolet radiation as compared with X rays. The latter produce a relatively high proportion of chromosomal alterations, while ultraviolet radiations produce mostly point mutations. Since point

mutations, as Stadler (1932) has pointed out, are merely those alterations in the germ plasma for which a mechanical chromosomal basis cannot be detected, they may actually consist of a whole assemblage of different physical and chemical changes, including rearrangements of the micellae of a colloidal system, of individual molecules, and of various rearrangements of rings, side chains, or even of individual atoms within the molecule. Since among the visible structural alterations of the chromosomes there is no close correlation between the size of the change and the magnitude of its genetic effect, there is no reason for believing that such a correlation exists on a submicroscopic level.

The obvious implication of the above remarks is that the nature of mutation is no longer a problem primarily of evolutionary biology or even of genetics per se, but of nuclear biochemistry. From now on, our new knowledge of mutation will come almost entirely from a better understanding of the physicochemical structure of chromosomes and of the biochemistry of gene action. The rapid advances being made in this field suggest that the evolutionist may not have very long to wait for the vital information he needs in order to understand the ultimate source of evolutionary change.

#### GENETIC EFFECTS OF MUTATIONS

Nevertheless, our understanding of the role of mutation in evolution will be greatly helped by a summary of the salient facts about the nature of the genetic effects of point mutations and about the rates at which they occur. And in discussing these genetic effects, we must always keep in mind this cardinal fact: the most direct, immediate action of genes is on the processes of development, and genes produce effects on the visible, morphological characters of the mature organism only through their influence on development. Similarly, mutations directly alter processes, and only indirectly, characters. The recent developments in biochemical genetics (Beadle 1945, 1946) have served particularly to emphasize this point.

With these facts in mind, we can classify mutations according to the number of different parts of the organism they affect and also according to the extent and nature of their effect on individual organs. We can find in the different mutations now known



all possible combinations of extent and intensity of effect, from those which profoundly affect all parts of the organism to those which have a hardly detectable effect on a single character.

The obvious limit in extent and magnitude of genetic effects is set by the lethal mutations, which alter one or more of the metabolic processes of the cell so radically that death results. Hardly less drastic are those mutations which, in addition to altering profoundly the vegetative parts, change the reproductive structures of the plant to such an extent that they are sterile in the homozygous condition. These produce plants which in the older morphological terminology were called teratological monstrosities. Typical examples are those given by Lamprecht (1945a,b) for *Pisum* and *Phaseolus*. For instance, the *unifoliata* mutant of *Pisum* produces entire or three-parted leaves instead of the pinately divided type usually found in peas; the sepals and the petals are transformed into leaflike structures bearing rudimentary ovules; and the carpel, although recognizable as such, is by no means normal (Fig. 11, left). In another mutant, *laciniala*, the margins of the leaflets are strongly toothed, and most of them

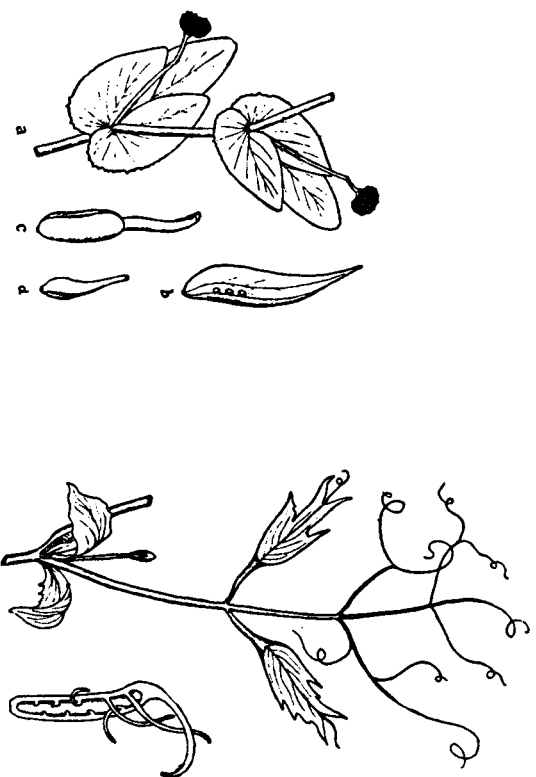


FIG. 11. The *unifoliata* (left) and the *laciniala* (right) mutants of the garden pea (*Pisum*). In the former diagram a pistilloid sepal (b), the pistil (c), and a pistilloid stamen (d) are shown in addition to a part of the plant; in the latter are shown a part of the plant plus the gynoeceum. From Lamprecht 1945.

are replaced by branching tendrils, while the pod is correspondingly modified (Fig. 11, right). Both of these types are completely sterile.

Of considerably greater interest are those mutations which produce a marked effect on many or all of the parts of the plant, but which do not result in weakness or sterility. Some of these are known as single gene differences in natural populations of wild or cultivated plants, where they presumably arose as mutations in the past and have since been designated as distinct species. Two good examples are the *oxyloba* gene of *Malva parviflora* (Kris-tofferson 1926) and the *sphaerococcum* gene of wheat, *Triticum aestivum* L. (Ellerton 1939, Sears 1947). The former produces a striking change in leaf shape, causing the lobes to be sharply pointed, and effects a similar change in the sepals. The *sphaerococcum* mutation produces a shorter, stiffer stem, a profuse tillering, small spikes or ears, practically hemispherical, inflated glumes, and small, round grains. Ellerton (1939) believed it to consist of a deficiency of a chromosomal segment, but Sears (1947) has demonstrated that this is not true, and has shown furthermore that it is a true recessive, since two *sphaerococcum* genes must be present before the character is expressed.

These two factors are typical of genes whose effect has been termed *pleiotropic*, that is, altering simultaneously several characteristics of the adult phenotype. There is good reason to believe, however, that this pleiotropy is not a peculiar property of certain genes, but results from the fact that certain developmental processes are important in the same way in several different organs, so that genes affecting these processes indirectly affect many characters. A mutant which illustrates this point is the *compacta* gene of *Aquilegia vulgaris* (Anderson and Abbe 1933). This gene affects all parts of the plant. It produces shorter, thicker stems, with the internodes much reduced in length; shorter petioles and rachises of the leaves; and a shorter inflorescence with the flowers on short, stout, erect peduncles. All these effects have been shown by Anderson and Abbe to be the result of precocious secondary thickening of the cell walls throughout the plant.

A series of genes which affect the leaves and also the parts of the flower, as well as those affecting only the latter, is described by



Anderson and DeWinton (1935) in *Primula sinensis* (see Fig. 12). They noted that those genes which affect leaf shape usually have a supplementary effect on the flower. Following the lead of Schultz, in his work on *Drosophila*, they suggest that these multi-

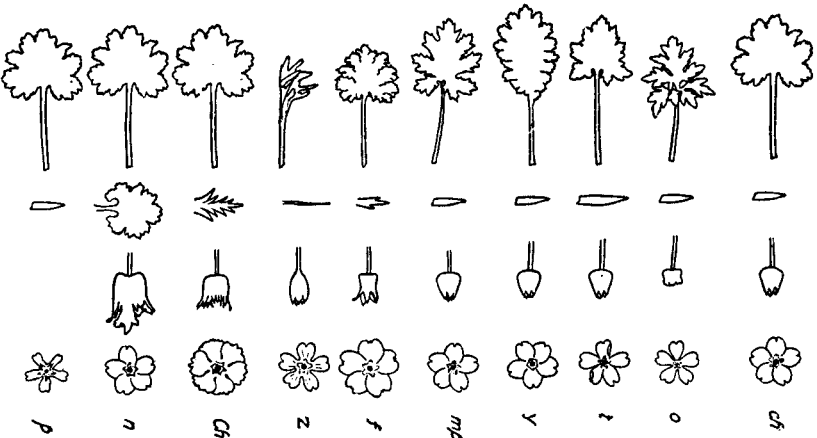


Fig. 12. Leaf, bract, calyx, and corolla of the "wild" type of *Primula sinensis* and of nine mutants affecting leaf and inflorescence shapes. All are shown on a wild type background except the calyx of "un," which is shown on a CHCh background. From Anderson and DeWinton 1935.

ple effects can be explained on the assumption that "each gene has its own job to do" in terms of affecting certain processes of growth. Crimp (ff), for instance, extends marginal growth on leaves, bracts, sepals, and corolla. Oak (oo) increases the sinuses on both corolla and leaf. In tongue, lateral growth is reduced in leaves, bracts, and corolla lobes. A gene with a similar type of

TABLE 1  
SUMMARY OF THE GENETIC COEFFICIENTS DIFFERENTIATING  
*Nicotiana glauca* FROM *N. langsdorffii*<sup>a</sup>

Genetic coefficients	Vegetative phase		Reproductive phase											Other coefficients of which this may be a further expression
	Axis	Leaf	Axis	Bract	Pedicel	Calyx	Tube	Throat	Limb	Stamen	Pistil			
(1) Cell size	x	x	x	x	x	x	x	*	x	x	x		(2)	
(2) Cell elongation	x	x	x	x	x	x	*	*	x	x	x		(2)	
(3) Geotropic response	x	x	x	x	*	x	?	?	x				(2)	
(4) Leaf-vein angles	x	x	x	x	x	x	x	x	*	*			(2)	
(5) Plastid color	x	x	x	x	x	x	x	x	*	*			(2)	
(6) Foliar periphery	x	x	x	x	x	x	x	*	*				(2)	
(7) Foliar base	x	x	x	x	x	x	x	*	*				(2)	
(8) Pollen color	x	x	x	x	x	x	x	*	*				(2)	
(9) Time of blooming	x	x	x	x	x	x	x	*	*				(2)	
(10) Scent	x	x	x	x	x	x	x	*	*				(2)	
(11) Inflorescence	x	x	x	x	x	x	x	*	*				(2)	

x = Organs in which the action of the genetic coefficient is evident.  
\* = The organ in which it can probably be measured most efficiently.  
<sup>a</sup> From Anderson and Ownbey 1939.

effect is the "petioled" gene of *Nicotiana tabacum* (designated as S in Setchell, Goodspeed, and Clausen 1922). This causes the leaves to have true petioles, without wing margins and with narrower, more acute or acuminate blades. In addition, the calyx lobes are narrower and more acute; the corolla lobes are more acuminate; the anthers are longer; and the capsule is narrower. Examination by the writer of developing leaves and flowers has shown that in "petioled" (S) plants the primordia of the leaves, when less than 1 mm long, are already narrower than are those of normal (s) ones. The "petioled" gene, therefore, apparently has the one effect of changing the shape of the primordia, but this affects each part of the plant in a somewhat different way, according to the developmental processes normally taking place in a particular

primordium. Genes with effects of this nature are probably responsible for the genetic coefficients described by Anderson and Ownbey (1939) in hybrids between *Nicotiana alata* and *N. langsdorffii*. Eleven such coefficients are listed, each of them affecting two or more different characters of the plant (Table 1). One of the most interesting of these is that affecting cell elongation. This causes *N. alata* to have narrower leaves, longer calyx lobes, a much longer style, a more pointed ovary, and particularly a longer corolla tube than *N. langsdorffii*. Nagel (1939) found that all these differences are due to the fact that *N. alata* inactivates auxin less readily than *N. langsdorffii*.

Genes with relatively restricted effects are also very common in plants. In *Nicotiana tabacum* the gene "broad" (A in Setchel, Goodspeed, and Clausen 1922), like "petioled," affects most strongly the basal part of the leaves, causing the wing margins of the leaves to become broader. But it does not alter appreciably the shape of the blade, and it has no visible effect on any of the floral parts. In contrast to those of "petioled," leaves of "broad" (A) plants are indistinguishable from those of normal or constricted (a) ones until they are well differentiated and about 2 cm long. This gene, therefore, acts on growth processes which take place relatively late in leaf development and have no counterpart in the development of the floral parts.

That there is no fundamental difference between these more "typical" genes and the "pleiotropic" ones described in the preceding paragraph is evident from the extensive studies of Stadler (unpublished) on alleles at the R locus in maize. At this single locus there is a long series of multiple alleles for anthocyanin color. Some of these affect the entire plant, others only the endosperm, and still others various combinations of plant parts, such as the coleoptile, the apex of the leaf sheaths, and the anthers. One could imagine a similar series affecting, not the formation of pigment, but of some growth-promoting substance. In such a series, the different alleles could affect the shape of one or several organs, depending on the developmental conditions under which they produced this growth substance. Thus, the apparent diversity of gene action may be largely a result of the complexity of the developmental processes taking place in any higher plant or animal. The same initial gene-controlled enzy-

matic process may give very different end results, depending on other processes going on at any particular stage of development.

The final set of genic effects to be considered are those which alter the phenotype very slightly, the so-called "small mutations." These were first described by Baur (1924) in *Antirrhinum majus* and its relatives, where they were estimated to occur at the extraordinarily high rate of one in ten gametes. East (1935b), by doubling the somatic chromosome number of a haploid plant of *Nicotiana rustica*, obtained a completely homozygous diploid. The first selfed generation from this plant was strikingly uniform, but in later generations considerable variability was observed. This was not measured quantitatively, but could not be explained except as a result of the occurrence of several mutations with slight phenotypic effect during the four generations of selfing. Lindstrom (1941) obtained quite a different result from selfing a completely homozygous strain of tomato obtained from a haploid. This line remained completely uniform for nine generations. The frequent occurrence of small mutations in *Drosophila* has been shown by a number of experiments of different types (Dobzhansky 1941, pp. 58-60). Their existence in natural populations of both plants and animals seems likely from the fact that most character differences between races and species show complex segregations in hybrids so that they appear to be governed by multiple factors (Tedin 1925, Münzing 1930a, East 1935a, Winge 1938, etc.). The building up of a character difference based upon multiple factors, as well as the development of a system of modifiers which alter the expression of genes with large effects, must be due to the occurrence and establishment in the population of many small mutations. The statement of Harland (1934) that "the modifiers really constitute the species" can be interpreted in no other way than that small mutations are the most important ones in building up the differences between species, as Harland showed clearly in a later, more detailed review (1936). The large body of evidence which has now accumulated compels us to agree with East that (1935a, p. 450) "the deviations forming the fundamental material of evolution are the small variations of Darwin."

The examples given above and many others like them answer effectively the question which has often been raised as to whether

mutations always produce abnormal or weakening effects or whether some of them may be considered the basic units of progressive evolutionary change. Mutant types like those cited in *Maba*, *Triticum*, and *Nicotiana* are in every way comparable to the individual character differences which separate natural subspecies and species. To be sure, the origin of these mutants was not actually observed, but the indirect evidence for their manner of origin is very strong. It is, however, true that most of the mutations appearing in artificial cultures of plants or animals, both spontaneously and under the influence of such agencies as X rays, produce phenotypes less viable than the original "wild" type. But as Dobzhansky (1941, p. 26) and many others have pointed out, this result is expected. The gene complement of any species is a complex system composed of many different units interacting in a balanced, harmonious fashion. It can be thought of as a complex machine which runs smoothly only when all its parts bear the correct relationship to each other, both in structure and in function. In such a machine, the number of changes that will improve the working of the whole is small, and the number that will injure or destroy its activity is very large. Furthermore, most modern species have been on the earth for thousands or even millions of generations, and during that time have been exposed to similar types of environment. During this time, therefore, one would expect most of the mutations which might improve the adjustment of the species to these environments to have already occurred several times and to have had plenty of opportunity to become incorporated into the genic complement of the species by natural selection, so that even occasional valuable mutations could be expected only in species exposed to a new type of environment. Under any hypothesis about the role of mutations in evolution we should expect most of these changes to be neutral or harmful to the species and only occasional ones to have even potential importance in evolution.

These hypotheses have recently been confirmed experimentally in a striking fashion. Gustafsson (1941a,b, 1947a) has by X radiation induced a large number of mutations in barley, of which the great majority reduce the viability or fertility of the plant. Among those without clearly deleterious effects, nearly all reduce the total yield of grain, and most of them produce other agri-

culturally undesirable characteristics. But out of the hundreds of mutations obtained and tested, about ten were found which either increase the yield or produce other valuable characteristics, such as stiffness of straw and earliness. These characteristics might be incorporated by hybridization into established high-yielding varieties. Gustafsson (1947a) mentions in addition the production by Granhall and Levan of a typical "defect" or "loss" mutation, namely, a yellowish-green chlorophyll mutation in flax, which nevertheless gave a higher yield of straw and a better quality of fiber than the variety in which it arose.

Another type of evidence of the occurrence of beneficial mutations was obtained by Dobzhansky and Spassky (1947) in *Drosophila pseudoobscura*. By means of a special technique, they produced strains of flies completely homozygous for certain particular chromosome pairs which were known to carry genes causing a reduction in vigor when in the homozygous condition. Seven strains of this type were isolated and bred for fifty generations. At the end of this period, five of the seven strains showed improvement in viability, and two of them were as viable or more so than flies carrying the corresponding chromosomes in the heterozygous condition. That these striking improvements were caused by new mutations was demonstrated by tests of the individual chromosomes concerned on a neutral genetic background. On the other hand, control stocks which were kept in a balanced heterozygous condition by means of a homologous chromosome containing an inversion and certain marker genes did not improve their viability during the same fifty generations of breeding, and in fact tended to deteriorate somewhat. This shows that if the genotype is artificially made subnormal and then bred in a normal environment, mutations which were being eliminated from the genetically normal population now have a high selective value and can therefore become established. Mutation, therefore, although it is the ultimate source of genetic variability in populations, acts only occasionally and under certain special conditions as a direct active agent in evolutionary change.

The foregoing discussion has been based on the assumption that mutation is at random, at least in respect to adaptation and to the origin of races, species, or phylogenetic trends in evolution.

The contrary assumption, that species, genera, or directed, "orthogenic" evolutionary lines are produced by means of special types of mutations, has been maintained by a number of workers, in recent times notably Goldschmidt (1940) and Willis (1940) in respect to special types of mutations; and Schaffner (1929, 1930, 1932) and Small (1946) in respect to orthogenetic trends. Criticisms of their opinions have been voiced by Wright (1941a,b), Dobzhansky (1941), Mayr (1942), Simpson (1944, pp. 150-157), and many others. These criticisms are in general valid. The arguments in favor of the importance of "systemic mutations, macromutations" and internally directed orthogenetic series are largely negative. Their proponents are unable to find conditions intermediate between intergrading, interfertile subspecies and sharply distinct species; or between the presence and the absence of some character of phylogenetic importance; or they cannot see the selective basis of certain evolutionary changes; and therefore they assume that these conditions do not exist. But such negative evidence is convincing only if it can also be shown that we know and understand fully all the facts about species formation or about the selective value of certain gene combinations. The cases presented by the authors mentioned above indicate no such omniscience.

There is no positive evidence whatever in favor of the occurrence of internally directed mutational changes which force the evolutionary trend of a line in any particular direction, as is postulated by adherents of the strictly orthogenetic concepts of evolution, such as Schaffner. Repeated observations and experiments on both spontaneous and artificially induced mutations in a great variety of organisms have shown that they are at random, at least in respect to the species differences and evolutionary trends which have occurred in the groups studied. It is highly unlikely that future experiments, at least in the higher animals and plants, will ever produce such positive evidence.

Positive evidence in favor of the importance in species formation of "large" mutations consists chiefly of the demonstration that mutations occur which produce certain character differences important for distinguishing species, genera, or families. Thus Goldschmidt (1940) stresses the importance in *Drosophila* of mutant flies with no wings or with four wings instead of the

two ordinarily present. A comparable mutant in plants is the *radialis* mutant of *Antirrhinum majus* (Baur 1924, Stubbe and Wettstein 1941), which transforms the two-lipped, zygomorphic corolla ordinarily found in snapdragons into a structure with radial symmetry and therefore characteristic of more primitive families. Numerous other examples are known, notably those described by Lamprecht (1939, 1945) in *Pisum*, *Phaseolus*, and other genera, and by Anderson and DeWinton (1935) in *Primula sinensis*.

The problem in connection with all these radical changes in a single character is whether they ever are or can be incorporated into the normal gene complement of a species. Darwin, in the *Origin of Species*, first expressed the opinion that they alter so drastically the relation between the individual and its environment that they must nearly always be a great disadvantage in competition with the normal condition, and most modern evolutionists agree with this opinion. This is undoubtedly true of the *radialis* mutation of *Antirrhinum*: the flowers of this mutation are so constructed that they cannot be visited by insects, and the anthers and stigma are so situated that pollen from the former cannot normally reach the latter, so that the mutation is sterile unless artificially pollinated. On the other hand, Stubbe and Wettstein (1941) have described other mutations in *A. majus* which produce equally drastic changes in the flower, but which are nevertheless fully fertile and viable. The most interesting of these is mut. *transcendens*, which reduces the number of stamens. This effect partly simulates the trends toward reduction in stamen number which have taken place in many genera of the family Scrophulariaceae, to which *Antirrhinum* belongs. Stubbe and Wettstein have suggested that in the evolution of the genera concerned, changes in the organization of the flower may have been caused originally by the establishment of mutations with large effects, and that the existence of multiple-factor inheritance in respect to these characteristics could have been acquired later through the establishment of modifier complexes, buffering or reducing the effect of the original mutations. Experiments to test this hypothesis are urgently needed, but although it may be true in many instances, there are nevertheless many phylogenetic trends in plants which are represented by so many transitional

stages among existing species that their progress through the accumulation of mutations with small effects seems most likely. Both "switch genes," with large phenotypic effects, and multiple factors or "polygenes," with small ones, have probably been important in the origin of new evolutionary lines in different plants, and their relative importance has probably varied in different groups. Furthermore, as Stubbe and Wettstein have pointed out, the two types of mutations are not sharply defined categories, but are connected by a continuous series of mutations with intermediate effects. Finally, as will be brought out in Chapter VI, even the most drastic of single mutations cannot possibly be considered as able to produce at a single step a new species or evolutionary line. This requires the establishment not only of complexes of genes affecting external morphology but also of genetically controlled isolating mechanisms.

#### RATES OF MUTATION

Although new mutations probably serve only rarely as the direct agents of evolutionary change, nevertheless the continuation of evolution obviously depends on the maintenance of a sufficiently high mutation rate. For this reason some knowledge of spontaneous as well as of environmentally controlled mutation rates is essential to an understanding of evolution.

Mutations do not by themselves produce evolution, but the mutation rate may under some conditions be a limiting factor. As has been emphasized by Dobzhansky (1941, pp. 34-42), spontaneous rates of mutation, though nearly always low, vary enormously from one race to another of the same species and from one gene locus to another of the same individual. In *Drosophila melanogaster*, the frequency of lethal mutations may be ten times as great in one strain as it is in another, and there is no reason to believe that the amount of variability in the rate of visible mutation is any less. And the data of Stadler in maize show that spontaneous mutation in such loci as R (color factor) may be more than 500 times as frequent as in other loci, like Wx (waxy). For this reason, mutation rates themselves must be looked upon as racial and specific characteristics subject to the same laws of evolutionary change as are the other morphological and physiological characteristics of the organism.

Since the epoch-making discovery of Muller that mutations can be induced in a high frequency by means of X rays, a very large number of experiments has shown that not only these radiations but also ultraviolet and infrared rays, as well as temperature shocks and some (though surprisingly few) chemical agents, can influence directly or indirectly the mutation rate. But these agents are effective only in very high, sublethal doses, and Muller (1930) has himself pointed out that only a fraction of 1 percent of the mutations occurring in natural populations can be accounted for by the radiation present in nature.

Of more interest to the evolutionist are the demonstrations of the effectiveness of certain chemicals, particularly mustard gas and related compounds, in producing mutations (Auerbach, Robson, and Carr 1947). Although the germ plasma may not be exposed very often to the action of such chemicals coming from outside, some of these mutations may be produced regularly or occasionally as by-products of metabolism within the cell. They may therefore be a cause of mutability produced by internal as well as by external agencies. At any rate, the hypothesis which seems most plausible at present is that mutations under natural conditions are usually caused, not by factors of the external environment, but by internal factors, either the slight physical or chemical instability of the gene or the action upon it of substances produced by the organism in which it is located.

Strong evidence in favor of such a hypothesis is provided by the examples of the increase in mutability of one gene through the effect of others with which it is associated. The best example of this was obtained by Rhoades (1938) in maize. The gene  $a_1$  causes absence of anthocyanin pigment in the endosperm. Normally it is very stable, but when a gene known as  $dt$ , lying in an entirely different chromosome from  $a_1$ , is replaced by its dominant mutant  $Dt$  ("dotted"), this mutant causes the gene  $a_1$  to mutate to its dominant allele  $A_1$ , which produces anthocyanin pigment in the cells of the endosperm and in other parts of the plant. The dominant mutant  $Dt$  is itself very stable and has no visible effect on the phenotype except to increase the mutability of  $a_1$ . A comparable example is that described by Harland (1937) in cotton. Both of the two related species, *Gossypium purpurascens* and *G. hirsutum*, have dominant genes for the occurrence of colored

spots at the base of the petals and corresponding recessives for the absence of this spot. If a plant of *G. purpurascens* homozygous for its dominant gene for petal spot ( $S^sS^s$ ) is crossed with a homozygous spotless ( $ss$ ) plant of *G. hirsutum*, the resulting hybrid, as expected, has petals with spots on most of its flowers, but evidently there is frequent mutation to the spotless condition. These mutations may occur in the vegetative growing point and produce branches on which all the flowers are spotless, or they may occur as late as the differentiation of the petal tissue itself, so that the spots contain streaks of colorless tissue. Pollen from flowers with spotted petals, when used on spotless plants of *G. hirsutum*, gives the expected 1:1 ratio of mutable heterozygous and spotless homozygous progeny; but when pollen was taken from spotless flowers of the same  $F_1$  plant, the resulting back-cross progeny with spotless *G. hirsutum* were all homozygous and spotless. This test, like a comparable one performed by Rhoades on the *Dt* mutant, shows that the changes induced are actually gene mutations.

The widespread occurrence of genetic factors affecting mutability has been strikingly demonstrated in the corn smut, *Ustilago zeae*, by Sakman, Kernkamp, King, and Martin (1943). This species includes an enormous number of haploid biotypes, many of which yield typical segregation ratios for visible characteristics when crossed with each other. Some of these biotypes are relatively constant, while others are highly mutable, and all sorts of intermediate conditions exist. In one cross, the four monosporidial haploid lines derived from a single zygote consisted of two which did not produce any sector mutants in 100 different flasks and two others which showed as many as 360 distinct sectors in 89 flasks. The mutants were of various morphological types, suggesting that the mutability factor concerned was a general one, affecting many different gene loci. In another cross between two mutable lines, segregation for mutability was also observed and was found to be linked with a factor determining the presence or absence of asexual sporidia, although recombination types, presumably crossovers, were occasionally found. Appropriate crosses between lines of the same kind with respect to variability and constancy make possible breeding either for increased constancy or for increased mutability.

Further examples of this sort are needed, but the evidence to

date suggests that mutation rates in nature are more frequently and more drastically altered by internal agents than they are by external agents. If this is the case, then the hypothesis of Sturtevant (1937) and Shapiro (1938), that mutation rates are themselves controlled by natural selection, becomes very plausible. These authors conclude that since in all well-established and adapted species most mutations will be harmful, selection will through the ages tend to lower the mutation rate in the genes of a species by establishing mutant genes which act as mutation suppressors. The behavior of the petal-spot gene of *Gossypium purpurascens* in the genetic environment of another species, away from many of the genes normally associated with it, supports this hypothesis. Other supporting evidence is provided by Gustafson's (1947a) studies of spontaneous and induced mutation rates in three different varieties of cultivated barley. The Golden variety, which "represents a very old pure line (isolated before 1900)," has the lowest rate of spontaneous mutation as well as of morphological mutations of various types induced by X rays. The two varieties Maja and Ymer, both of which, although they are now essentially homozygous, are the products of relatively recent hybridization and have higher frequencies of both spontaneous and X ray-induced morphological mutations. One may conclude that the history of Golden barley and of other old varieties of cereals has included many generations of artificial selection for constancy, which has involved in part the establishment of gene combinations reducing the mutation rate. The breaking up of these combinations by intervarietal hybridization is responsible for the increased mutation rates in the newer varieties. Similar evidence in *Drosophila* has been obtained by Berg (1944). She found that small populations of *D. melanogaster* found in a mountain valley near Eryvan, Armenia, where conditions are liminal for the species and selection pressure must be severe and rigid, had a relatively low mutation rate, while the larger populations occurring in more favorable sites had more rapid mutation rates both for lethal mutations and for morphological mutations at particular loci, such as yellow.

The evidence presented above suggests that the facts about mutations and mutation rates which are most important for studies of evolution are likely to be obtained in the future chiefly

from the as yet hardly developed study of comparative genetics. Mutation is not a completely autonomous process, but is integrated with the other genetically controlled physiological processes of the organism. Like them it is under the influence of natural selection and of other forces controlling the frequency of genetic types in populations.

## CHAPTER IV

### Natural Selection and Variation in Populations

SINCE ITS EXPOSITION in the classic work of Darwin, the theory of natural selection has had a tortuous history. In the half-century following the publication of the *Origin of Species*, this theory was regarded as the cornerstone of evolutionary biology, and nearly all of the research on evolutionary problems involved studies either of adaptation and selection or of the phylogenetic relationships between organisms. The proponents of selection undoubtedly went to unjustified extremes in their attempts to show the all-inclusiveness of this principle. In the early part of the present century, however, the prestige of the selection theory declined until many biologists regarded it not only as a relatively unimportant factor in evolution but in addition as a subject not worthy of study by progressive, serious-minded biologists. The reasons for this decline lay partly in the excesses of speculation committed by the extreme selectionists of the previous generation. But a more important cause was the impact on biological thinking of two fundamental discoveries in genetics. The first of these was the discovery of mutations by De Vries, and the second was the demonstration by Johansen that artificial selection is ineffective in a pure line of completely homozygous organisms. These two workers and their followers maintained that all true evolutionary progress comes about by sudden mutational steps, which create at once a new race or species. Selection was regarded as a purely negative force, eliminating the new variants which are obviously unfit, but not taking part in the creation of anything new. The prestige of these two geneticists, as well as the rapid strides being made by the science they helped to found, caused their views to be accepted widely and often uncritically, so that the mutation theory was often looked upon as a modern substitute for the largely outmoded hypothesis of Darwin.