

necks" involving temporary reductions in population size in the origin of the isolating barriers which separate species. These might be expected to occur more frequently in plants than in animals, since animals could migrate more easily to escape temporary vicissitudes of the climate. It is perhaps noteworthy that the nearest approach among plants to the variation patterns found in most of the higher animals is found among forest trees,

which have large populations and a maximum mobility of the gametes through wind transportation. Here again, a comparative study of habits of ecology, population size, and the nature of species and the genetic system in various orders of insects would doubtless be very enlightening. It may be that differences in these respects as great as those found between the higher plants and the vertebrate animals exist between different orders of insects. Nevertheless, comparisons between the genetic systems of animals and plants and their relation to the types of evolution found in the two kingdoms will undoubtedly be very fruitful subjects for future research.

CHAPTER VI

Isolation and the Origin of Species

ONE OF THE MOST important principles which has emerged from the recent studies of evolution is that the terms "evolution" and "origin of species" are not synonymous, as implied by the title of Darwin's classic. The foundation of this principle is the more precise, objective concept of the nature of species which has been obtained through more careful systematic and particularly cytogenetic studies of interspecific differences and of the barriers between species (Clauen, Keck, and Hiesey 1939, Dobzhansky 1941, Chap. XI, Mayr 1942, Chap. VII). The new definitions of the species which have been based on these studies are numerous; a different one can be found in each of the three publications cited in the previous sentence, while the group of essays compiled by Huxley (1940) contains nine more. Comparing these definitions, however, one is struck, not by their diversity, but by the large common ground of agreement between them. All of them stress the importance of genetic and morphological continuity within species, and recognize at the same time that a species may include within its limits an array of morphologically and physiologically diverse genetic types. They also agree that the boundaries between the species of sexually reproducing organisms are real, objective phenomena, and that they are produced by isolating mechanisms which prevent or greatly restrict the exchange of genes between the members of different species. The common ground of agreement between these definitions may be expressed as follows. In sexually reproducing organisms, a species is a system consisting of one or more genetically, morphologically, and physiologically different kinds of organisms which possess an essential continuity maintained by the similarity of genes or the more or less free interchange of genes

between its members. Species are separated from each other by gaps of genetic discontinuity in morphological and physiological characteristics which are maintained by the absence or rarity of gene interchange between members of different species. The above sentences are not to be construed as this author's definition of a species, since several different species definitions are possible within the framework of their meaning.

In the light of this concept, the processes involved in "descent with modification," to use Darwin's classic phrase, can be shown clearly to apply to differentiation within species, as well as to the further divergence of species and higher categories once they have become separated from each other. The processes peculiar to the origin of species are those involved in the building up of isolating mechanisms which restrict the interchange of genes between different species. One purpose of this chapter is to show that the processes responsible for evolutionary divergence may be entirely different in character and genetically independent of those which produce isolating mechanisms and, consequently, distinct species.

A COMPARISON OF DIFFERENCES WITHIN SPECIES AND BETWEEN SPECIES

The first question that arises is whether the characteristics of external morphology and physiology which distinguish species are different in kind from those between different races or subspecies of the same species, or whether they differ only in degree. The answer to this question is unequivocal, and the data from cytogenetic studies support the opinion held by most systematists. The differences between closely related species are nearly all duplicated by or paralleled by differences between races or subspecies of a single species. In many instances the traits which characterize genera or even higher categories can be found to vary within a species.

Some examples to illustrate this fact have already been presented in the discussion of ecotype differentiation in Chapters I and II. In *Potentilla glandulosa*, for instance, the differences between ecotypes include characteristics of the size and shape of sepals and petals, and the size, shape, and color of the achenes,

which are quite similar to characters used in other subgenera for the separation of valid species. In *Crepis*, Babcock and Cave (1938) found that the species previously classified by systematists as *Rodigia communata* is able to exchange genes freely with the widespread species *Crepis foetida*. Thus, the character difference which had previously been assumed to be of generic significance, namely, the presence or absence of paleae on the receptacle, was found to be an actual interracial difference within the limits of a single species. A similar example is that of *Layia glandulosa* var. *discoidea*, described by Clausen, Keck, and Hiesey (1947). This form is so strikingly different from its relatives in characteristics generally assumed to be of generic or even tribal significance that its original collectors were hesitant to place it in the tribe Madiniae. But when crossed with the well known *Layia glandulosa* it formed fertile hybrids, which in the second generation yielded a great array of vigorous and fertile individuals, segregating for the characteristics of involucral bracts and ray florets, which are commonly used as diagnostic of genera and tribes in the family Compositae. Further examples of differences which have commonly been assumed to separate species, but may be only of racial or subspecific significance may be found in the discussion in Chapter II concerning the variation patterns within the genera *Aquilegia* and *Quercus*. From his hybridizations in the genus *Salix*, Heribert-Nilsson (1918) found that in their genetic basis, interspecific differences in that genus differ from intervarietal ones only in magnitude and degree of complexity.

The objection might be raised here that the examples given in the above paragraph apply only to the diagnostic characters which have provided convenient "handles" for the systematist, not to the perhaps more "fundamental" physiological and biochemical differences between species. In answer to this objection, however, we can refer to the experimental studies which show that different races or ecotypes of the same species may have entirely different systems of reaction to their environment. *Potentilla glandulosa* subsp. *typica*, which grows actively throughout the year, differs radically in its physiological reactions from the alpine subsp. *nevadensis*, which even in the environment of *typica* remains dormant for the winter months. But these two subspecies are

completely infertile, are connected by a whole series of naturally occurring intermediate types, and the physiological differences between them show genetic segregation of exactly the same type as that which characterizes the recognizable differences in external morphology. Another important physiological difference, namely, the reaction to photoperiodism, is most often of specific value, but in *Solidago sempervirens* (Goodwin 1944), *Bouteloua curtipendula* (Olmsted 1944), and probably in other species it is a characteristic difference between races of the same species. Self-incompatibility ("self-sterility") and self-compatibility ("self-fertility") usually separate species, but in *Potentilla glandulosa*, *Antirrhinum majus* (Baur 1932), and several other species self-incompatible and self-compatible races exist within the same interfertile population system.

The same may be said of biochemical and serological differences. In general, these are more profound when species are compared than when comparison is made between races, but the difference is only in degree. Differences exist within species of *Dahlia*, *Primula*, *Papaver*, *Streptocarpus*, and other genera in the biochemistry of their flower pigments (Scott-Moncrieff 1936, Lawrence and Scott-Moncrieff 1935, Lawrence, Scott-Moncrieff, and Sturges 1939), of tomato in vitamin content, and of maize in aleurone and other chemical substances in the grain. Differences in antigenic properties have been used to determine the relationships between species, genera, and families both of animals and of plants (Irwin 1938, Mez and Siegenspeck 1926), but the same type of difference can be found on a smaller scale between races of the same species, as is evident from the work of Arzt (1926) on barley (*Hordeum vulgare* and *H. spontaneum*), as well as the studies of blood groups in man and of the still more complex serological differences between races of cattle (Owen, Stor-mont, and Irwin 1947). That morphological and biochemical differences should run parallel to each other is of course what one would expect, since differences in external morphology are simply the end products of different biochemical processes occurring in the development of the individual.

Further evidence for the essential similarity between inter-racial and interspecific differences is found in the example of

"cryptic species," that is, of population systems which were believed to belong to the same species until genetic evidence showed the existence of isolating mechanisms separating them. A typical example in genetic literature is that of *Drosophila pseudoobscura* and *D. persimilis* (Dobzhansky and Eppling 1944). In plants, two of the best examples are the complex of *Hemizonia* (or *Holocarpha*) *virgata-heermannii-obconica* (Keck 1935) and *Crepis neglecta-fuliginoosa* (Tobgy 1943). Both of these are in the family Compositae. In regard to the former, Keck writes as follows in his description of a new species, *H. obconica*, ". . . its distinctive characteristics were not observed clearly until cytological studies had shown that there were two species in the *H. virgata* complex. *Hemizonia obconica* is a species with a haploid chromosome number of 6 (like *H. heermannii*), while in *H. virgata* the number is 4. The species do not form fertile hybrids whether growing side by side in nature or as the result of artificial garden crossings." The differences in external morphology, as well as in physiological characteristics of growth and development, between *Crepis neglecta* and *C. fuliginoosa* are in every way comparable to those between different subspecies of certain other species, such as *C. foetida* (Babcock 1947). In both of these examples, the specific status of the entities concerned is based largely on discontinuities rather than large differences in morphological characteristics, and particularly on numerical and structural differences in the chromosome complements which cause the sterility of the F_1 hybrids. Such obvious chromosomal differences are not, however, essential accompaniments of the differentiation between species, as will be evident from examples presented later in this chapter.

The concept that species differences are of a different order than interracial differences within the species has been advocated most strongly in recent times by Goldschmidt (1940). The examples which he gives are drawn mostly from zoological literature, with particular emphasis on his own experience with the moth genus *Lymantria*. Mayr, however, has pointed out (1942, pp. 137-38) that in this example Goldschmidt dealt with only three of the numerous species of this genus, and that these three species are related most closely, not to each other, but to different ones of the species not studied by Goldschmidt. *Lymantria*, there-

fore, is not well enough known as a genus so that any conclusions can be drawn about the nature of the species forming processes in it. The only plant example which Goldschmidt cites in this particular discussion is that of *Iris virginica* and *I. versicolor*, quoted from Anderson (1936b). This was certainly an unfortunate choice. As is pointed out below, in Chapter IX, *I. versicolor* is an allopolyploid which contains the chromosomes of *I. virginica* combined with those of *I. setosa* var. *interior* or a related form. These species, therefore, represent a type of evolution which has nothing to do with the origin of species on the diploid level. Elsewhere in his book Goldschmidt has stated that species represent different "reaction systems," but this term is nowhere clearly defined. When applied to species, it can have only one meaning which agrees with the factual evidence. This is that members of the same species are genetically compatible, in that they can intercross freely and produce abundant fertile, vigorous, segregating offspring; while members of different species often (but not always) react with each other in such a way that hybrids between them either cannot be obtained or are incapable of producing vigorous, fertile progeny.

This similarity between interracial and interspecific differences carries with it the implication that species may be derived from previously existing subspecies. The converse statement, that all subspecies are destined eventually to become distinct species, is, however, very far from the truth. The isolating barriers which separate species arise only occasionally, and until they appear the different subspecies of a species will be firmly bound to each other by ties of partial morphological and genetic continuity.

A further statement which may safely be made on the basis of existing knowledge is that many subspecies may become species without any further divergence in morphological characteristics. If, for instance, environmental changes should wipe out all of the populations of *Potentilla glandulosa* except those inhabiting the coast ranges and the high Sierra Nevada of California, then the surviving populations would have all the external characteristics of two different species. Furthermore, if the same changes should bring about the establishment in one of these population systems of chromosomal differences which would cause it to produce sterile hybrids with its relatives, then two species would have been

differentiated out of one preexisting specific entity. At least some differentiation and divergence in morphological and physiological characteristics is the usual and perhaps the universal accompaniment of the process of species formation. But this divergence need not be different in kind or greater in degree than that responsible for the differentiation of races and subspecies within the species.

The critical event in the origin of species is the breaking up of a previously continuous population system into two or more such systems that are morphologically discontinuous and reproductively isolated from each other.

THE EVOLUTIONARY SIGNIFICANCE OF SPECIES FORMATION

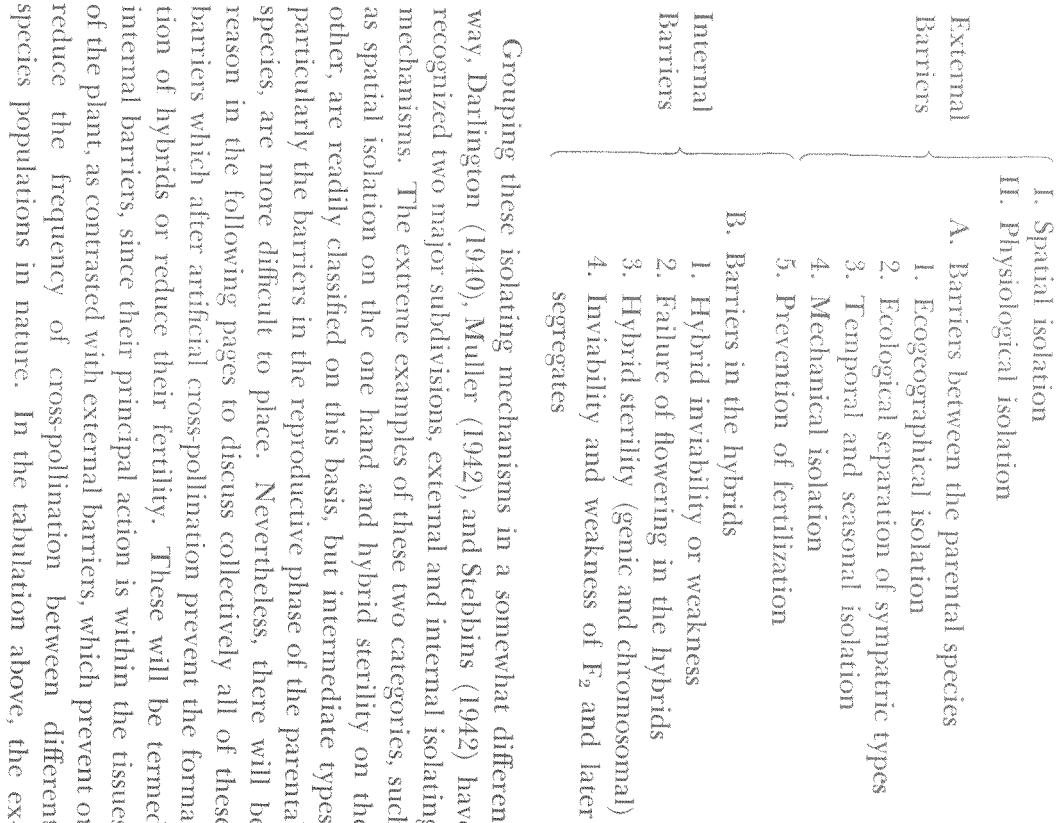
The characterization given above of the species-forming processes might seem at first glance to relegate speciation to a comparatively minor role in the drama of evolution. But this is by no means the case. As Muller (1940) has pointed out, the segregation of a previously interbreeding population system into two reproductively isolated segments tends to restrict the supply of genes available to each of these segments and tends to canalize them into certain paths of adaptation. Evolutionary specialization is therefore greatly furthered by the process of speciation.

This concept gains further importance when we realize that by far the greatest proportion of the diversity among living organisms reflects, not their adaptation to different habitats, but different ways of becoming adapted to the same habitat. Biological communities consisting of scores or hundreds of different species of animals and plants can exist in the same habitat because each species exploits the environment in a different way than its associates. The specificity of each different organism-environment relationship is maintained by the failure of the different species in the same habitat to interbreed successfully (Muller 1942). Speciation, therefore, may be looked upon as the initial stage in the divergence of evolutionary lines which can enrich the earth's biota by coexisting in the same habitat.

TYPES OF ISOLATING MECHANISMS

A classification of the different isolating mechanisms which form the barriers between species is given by Dobzhansky (1941,

p. 257). This has been somewhat simplified and adapted to the phenomena found in plants by Brieger (1944a), but his system has some undesirable characteristics, such as the failure to recognize the fundamental distinction between purely geographical or spatial isolation and the various types of reproductive isolation. The following classification is intended to combine the simplicity introduced by Brieger with the more advantageous arrangement established by Dobzhansky.



Grouping these isolating mechanisms in a somewhat different way, Darlington (1940), Muller (1942), and Stebbins (1942) have recognized two major subdivisions, external and internal isolating mechanisms. The extreme examples of these two categories, such as spatial isolation on the one hand and hybrid sterility on the other, are readily classified on this basis, but intermediate types, particularly the barriers in the reproductive phase of the parental species, are more difficult to place. Nevertheless, there will be reason in the following pages to discuss collectively all of these barriers which after artificial cross-pollination prevent the formation of hybrids or reduce their fertility. These will be termed internal barriers, since their principal action is within the tissues of the plant, as contrasted with external barriers, which prevent or reduce the frequency of cross-pollination between different species populations in nature. In the tabulation above, the ex-

ternal barriers are those under the headings I and II A, 1-4, while the internal ones are listed under II A, 5, and II B, 1-4.

SPATIAL AND ECOLOGICAL ISOLATION IN RELATION TO SPECIES FORMATION

As a generalization, purely spatial or geographical isolation is, as Dobzhansky points out, on an entirely different plane from all the different reproductive isolating mechanisms. When applied to specific examples, however, the reality of this distinction is vivified by two facts. In the first place, this type of isolation may persist over long periods of time without causing the isolated populations to diverge from each other enough to become recognizably distinct or even different. Species with disjunct distributions are well known to phytogeographers, and numerous examples are given by Ternald (1929, 1931), Cain (1944), and many other authors. Furthermore, as is pointed out in Chapter XII, the

nature of many distributions strongly suggests that some of these disjunct segments of the same species have been isolated from each other for millions of years. There is some reason for believing, therefore, that geographic isolation alone often does not result even in the formation of subspecies. In continental areas, this type of isolation is important chiefly in conjunction with the divergent trends of selection produced by different ecological factors. In small populations, which are particularly frequent on oceanic islands, spatial isolation is the usual precursor to divergence in nonadaptive characters by means of genetic drift or random fixation.

Furthermore, geographically isolated races, as well as allopatric species, are usually separated from their relatives in adjacent regions by ecological barriers as well as by geographic ones. This is due to the fact that different geographic areas usually have different climatic and ecological conditions. In the great majority of examples of races, subspecies, or species which are kept apart because they live in different regions, the question may be justly asked if distance is the primary isolating factor, or if the initial separation and divergence has not been due to the response of the ancestral population to the selective effect of ecological differentiation in various parts of its originally more uniform environment. Most geographical races, therefore, may have arisen

primarily as ecotypes, and their separate distributions may be an incidental secondary result of this ecotypic differentiation.

In many groups of plants, particularly those with very effective methods of seed dispersal, this distinction between strictly geographical isolation and eogeographical isolation is of more than academic interest. For instance, a species of grass, pine, or goldenrod may be divided into two subspecies, one occurring in a more northerly area in a relatively cool, moist climate, and the other farther south in a warm, dry region. If one plots the distribution of the two subspecies on a map, one gains the impression that the isolation between them is primarily geographical. But since the seeds of plants can be borne by the wind or by animals for distances of scores or hundreds of miles, the seeds of such a northern subspecies would not infrequently settle in the territory occupied by a southern one, or vice versa. However, such seeds would not be likely to grow into adult plants, and so to transfer genes from one subspecies to another. This would be prevented by strong adverse selection pressure in the critical seedling stage.

A concrete example of such a situation is given by Baur (1932). *Antirrhinum glutinosum* is found in the Sierra Nevada of southern Spain at altitudes ranging from 800 m to 2,800 m on high mountain slopes which are snow-covered until late in May. Plants taken from the higher altitudes are highly frost resistant and possess a spreading habit which in nature causes them to be closely pressed against the cliffs on which they grow. Although all the plants occurring in nature had this growth habit, and the condition was retained when they were grown under cultivation, their offspring segregated strongly for this condition as well as for frost resistance, indicating heterozygosity of the wild plants for these two highly adaptive characteristics. Two conditions of the habitat of these *Antirrhinum* forms account for this situation. The seedling individuals possessing frost susceptibility and upright growth habit must be quickly eliminated by selection at the high altitudes. The maintenance in the high montane segment of the population of the genes for these strongly disadvantageous characteristics could be accomplished by the continual transfer from lower altitudes of gametes bearing them. Baur observed large numbers of small butterflies borne by the wind from the lower altitudes over the crest of the mountains without damage, and

therefore concluded that the transfer of pollen over the necessary distance by these or other insects could occur.

In such examples the two subspecies are kept apart, not by the inability of individuals to cross the geographical barrier separating them, but by the ecological factors which keep them from reaching maturity when they do so. The same arguments apply to related allopatric species.

SPATIAL AND ECOGEOGRAPHIC ISOLATION AS SPECIES-FORMING BARRIERS

By far the commonest type of isolation in both the plant and the animal kingdom is that resulting from the existence of related types in different geographical regions which differ in the prevailing climatic and edaphic conditions. Numerous examples of this type of isolation have already been given in Chapters I and II. If the ranges of the types thus isolated from each other come in contact at any point, and if no other isolating mechanisms exist, then intergrading will occur along these points of contact, and the populations concerned can be regarded as subspecies. But if the related populations are separated from each other by large gaps in which no related forms are found, and if the habitats they occupy are widely different ecologically, then the specific status of these forms is subject to very different interpretations, depending on the concepts held by different biologists.

An example of this situation is found in the genus *Platanus*. The two best-known species of this genus, *P. occidentalis* and *P. orientalis*, occur respectively in the eastern United States and the eastern part of the Mediterranean region. They are markedly different and distinct both in vegetative and in fruiting characteristics, so that Fernald (1931) considers that they represent "sharp specific differentiations." Nevertheless, the artificial hybrid between them, *P. acerifolia*, is vigorous and highly fertile and has perfectly normal meiosis (Sax 1933). The chief isolating mechanism separating *P. occidentalis* and *P. orientalis* is, therefore, the great difference between their natural ranges. This isolation is not merely geographic. The climates prevailing in the eastern United States and the Mediterranean region are so different that types adapted to one of these regions will rarely, if ever, grow spontaneously in the other. Under natural conditions, therefore,

these two species could not grow together spontaneously and exchange genes. They are sharply separated by barriers of ecological isolation, even though these are not evident at first glance.

Further studies of hybrids between widely allopatric species will probably reveal that the situation found in *Platanus* is not uncommon in the higher plants. In addition, there are doubtless many examples of generally recognized allopatric species which are sharply discontinuous from each other in morphological characteristics, but occupy regions which have similar climates and are ecologically equivalent. One such example is *Catalpa ovata* and *C. bignonioides*. The former of these two species is found in China and the latter in the eastern United States. These two regions have such similar climates and floras that species native to one of them could probably thrive in the other. Smith (1941) has found that the artificial hybrid between these two species is fully fertile. If, therefore, the geographic barrier between them were removed, they would very likely revert to subspecies of a single species.

The situations represented by *Platanus* and *Catalpa*, as well as many others in which geographic, ecological, and other types of isolation are combined in various degrees, present serious difficulties to the formulation and application of any precise biological species definition. If two sets of populations are ecologically similar and form fertile hybrids, so that they are separated only by spatial isolation, then they cannot be considered as reproductively isolated, and according to the strict application of any of the species concepts given earlier in this chapter they should be placed in the same species, no matter how different and distinct they are in external morphology. On the other hand, if two population systems are sympatric, but rarely or never form hybrids in nature because of their widely different ecological preferences, then they are often considered as distinct species, even though artificially produced hybrids between them are vigorous and fertile (Dobzhansky 1941, pp. 377-378). In order, therefore, to make valid inferences as to the specific status of allopatric, as well as sympatric, population systems, one must determine not only whether they can cross and produce fertile hybrids under the optimum conditions of a cultivated garden plot but, in addition, whether they could coexist in the same territory and hybridize under natural conditions.

Mayr (1942, p. 121) has suggested that in the case of allopatric forms which cannot be hybridized because they cannot be reared under domestication or cultivation, the decision as to whether they are distinct species or merely subspecies can be made by means of inferences. The amount of difference in characters of external morphology between the allopatric forms in question is compared with that between other related forms which are sympatric or at least overlapping in distribution. If this difference is about the same as that found between adjacent and intergrading subspecies, then the isolated, allopatric populations are also regarded as subspecies, but if the difference is greater, they are considered to be distinct species. In order to make such inferences in the case of allopatric populations of plants, one would have to take into account at least five sets of independent variables. These are hybrid sterility, hybrid inviability, cross-incompatibility, seasonal isolation, and ecological isolation. No one of these is closely correlated with morphological differences, as will be evident from numerous examples given later in this chapter. Obviously, therefore, in plants such inferences are very hazardous and unreliable. These considerations lead us to the conclusion that for a large proportion of plant groups, particularly the hundreds of species of woody plants of tropical distribution, we cannot hope to apply within the foreseeable future any species concept based entirely on reproductive isolation. This leaves only three alternatives open to us. The first is to have two species concepts. One of these, the strictly biological one based on reproductive isolation, would have to be reserved for those groups in which experimental hybridizations have been made or in which the ranges of the species and races overlap to a sufficient extent so that the amount of intergradation or hybridization between them can be determined by studies of naturally occurring populations. The second species concept, which would have to be applied to all groups not suited to experimental studies and with their species and races largely allopatric, would be a much more general and admittedly artificial one. The fact would have to be recognized that the species in these two series of plant groups are not biologically comparable entities. All studies of plant distribution and of the relative amount of species formation in different plant groups would have to take into account the existence of these two differ-

ent species concepts. This would be a most unsatisfactory situation from every point of view.

The second alternative would be to recognize that at any given moment in the evolutionary time scale, reproductive isolation is important in keeping distinct only those populations which are sympatric or which overlap in their distributions. If subjected to markedly different environments, allopatric populations can without the aid of any additional isolating mechanisms diverge to an extent sufficient to make them entirely distinct from each other in several characteristics. This degree of divergence could be accepted as the attainment of full specific status.

This principle of divergence to the point of sharp discontinuity in several characteristics as a species criterion could be applied to allopatric populations of woody plants in a reasonably precise and systematic fashion by the use of population studies as well as by the usual methods of systematics. In practice, the principle would be applied about as follows. Population A has been recognized by taxonomists as a woody species endemic to Mexico and sufficiently widespread so that millions of trees of it exist. Population B is found in Colombia and Venezuela and is likewise widespread. By studying quantitatively population samples from all parts of the area of distribution of both populations, the systematist could determine with some degree of accuracy the range of variation in each population of all the morphological characteristics by which the two populations might be expected to differ. Once this was done, he could estimate the probability that population B could produce by gene recombination individuals identical in all of their recognizable characteristics with at least some of the individuals present in population A. If this probability were high, or if these similar individuals were found in even a fraction of one percent of the hundreds or thousands studied, then populations A and B would have to be considered subspecies, no matter how different from each other their typical representatives were. But if in two or three genetically independent characteristics the range of variation in population A were entirely separate and distinct from that in population B, so that an individual of B could never be expected to match one of population A, then the two populations could be recognized as distinct species.

The third alternative would be to apply rigidly the criteria set

up by Clausen, Keck, and Hiesey (1939), and to recognize as distinct species only those population systems which are separated from each other by internal isolating barriers, so that artificial cross-pollination under cultivation fails to produce hybrids or yields hybrids of reduced viability or fertility. This alternative has the practical difficulties mentioned in connection with the first alternative, but in an accentuated form. As will be pointed out later in this chapter, the final test of fertility of a hybrid in some cases involves raising to maturity progeny of the second generation, and our present knowledge suggests that examples of isolating barriers which do not become evident until the second generation may be particularly common in long-lived woody species, in which a single experiment of this type may occupy a whole lifetime. Furthermore, population systems are being discovered in which two related strains may yield highly sterile hybrids when crossed with one another, but may be able to exchange genes via a third strain, with which both of them can form partly or wholly fertile hybrids. The example of *Oryza sativa*, the cultivated rice, is discussed later in the chapter, and others are known in *Bromus* (Stebbins, unpublished) and *Layia* (J. Clausen, oral communication). The discussions in this chapter of the genera *Aquilegia*, *Quercus*, *Ceanothus*, *Vaccinium*, the family Orchidaceae, and many other groups will make the fact evident to the reader that the strict application of the scheme of Clausen, Keck, and Hiesey would make necessary a drastic revision of the species in many genera as they are now recognized by most working systematists. And the facts mentioned above indicate that whatever might be the theoretical advantages of such revisions, attempting to carry them out under the present state of our knowledge would be totally impracticable and would produce more confusion than order in the taxonomic system as a whole. The great amount of revision which would be necessary to make some groups conform to this system is evident from examples such as that of Osborn (1941), who showed that a hybrid between *Cupressus macrocarpa* and *Chamaecyparis nootkatensis*, produced in cultivation, is highly fertile and yields seedlings which segregate strongly for the parental characteristics. On the basis of this evidence, the suspicion arises that under the scheme of Clausen, Keck, and Hiesey all of the species of *Cupressus* would have to be united with those of *Chamaecyparis* into a single species.

Which of these alternatives is the most desirable would have to be left to the decision of competent systematic workers, trained in modern methods and concepts. Our present state of knowledge does not confirm the opinion, often given by experimental biologists, that only individuals are real and that the species is a purely man-made concept. But it nevertheless is true that there are a number of equally real biological situations to which the traditional concept of the species may be applied. In the opinion of the present writer, the principal task of the experimental taxonomist and the evolutionist should be to study these situations and to spend as little time as possible discussing the definition and application of terms. As our knowledge of the biological facts becomes more complete, we shall gradually achieve a firmer basis, consisting of a large fund of common knowledge, on which to erect our species concept. Until then, the wisest course would seem to avoid defining species too precisely and to be tolerant of somewhat different species concepts held by other workers. The one principle which is unavoidable is that species are based on discontinuities in the genetic basis of the variation pattern rather than on the amount of difference in their external appearance between extreme or even "typical" individual variants.

If we accept this latitude in our species definitions, then we can recognize the existence as species isolating mechanisms of purely spatial isolation, strictly ecological isolation of sympatric forms, or various combinations of these two isolating factors. And the latter are by far the most common in nature.

ECOLOGICAL SEPARATION OF SYMPATRIC TYPES

Ecological isolation of sympatric species is, according to Mayr (1942, 1947), rather uncommon in animals. In plants, on the other hand, several examples are known. The various species of *Quercus* discussed in Chapter II are excellent ones. Another genus in which ecological isolation plays an important role is *Ceanothus*. This genus of shrubs contains many species in California, some of which present considerable difficulties to the systematist. Hybridization between recognized species is not uncommon both in nature and in gardens where various species are cultivated as ornamentals (McMinn 1942, 1944). All the species have the same number of chromosomes ($n = 12$, McMinn

1942) and similar chromosome complements; meiosis in interspecific hybrids is often quite regular, and many of the hybrids are highly fertile. Nevertheless, some of these species are sympatric over the entire extent of their geographic ranges and remain in general distinct from each other.

Typical examples are *Ceanothus thyrsiflorus*, *C. papillosus*, and *C. dentatus* in the central coast ranges of California. *C. thyrsiflorus* is a tall, vigorous shrub with large leaves and ample panicles of usually pale blue flowers, common on hillsides with relatively good conditions of soil and moisture. *C. papillosus* and *C. dentatus* are smaller and have much smaller leaves with very distinctive shapes and indumentum and small inflorescences of deep blue flowers. They grow on more exposed, drier sites, often with poor or shallow soil. They are more local in their occurrence than *C. thyrsiflorus*, and although the restricted range of *C. dentatus* is entirely included within the geographic distribution of the other two species, no locality is known to the writer which supports both *C. papillosus* and *C. dentatus*.

Evidence that the isolation between *C. thyrsiflorus* and *C. dentatus* is chiefly ecological is provided by their behavior at one locality in which they grow together and in which ecological conditions have been disturbed by the hand of man. On the northwest side of the Monterey Peninsula is a hillside traversed by several shallow gullies and low ridges, on the latter of which the soil is shallow and poor. The normal cover of this hillside is a dense stand of *Pinus radiata*, *P. muricata*, *Arctostaphylos tomentosa*, and *A. hookeri*, among which are mingled scattered shrubs of other species, including *Ceanothus thyrsiflorus* in the gullies and *C. dentatus* on the exposed rock outcrops of the ridges. Along this hillside have been cut several firebreaks or roadways, from which most of the pines and manzanitas (*Arctostaphylos*) have been removed. These have grown up to a luxuriant second growth, in which *Ceanothus* is predominant. On the better soil near the gullies, the species is *C. thyrsiflorus*, while the rocky outcrops on the crests of the ridges support the prostrate, small-leaved species, *C. dentatus*. The flanks of the ridges are occupied by intermediate types, which include every conceivable intergradation between and recombination of the characters of *C. thyrsiflorus* and *C. dentatus*. That these intermediates are of hybrid

origin is evident from the fact that seedling progenies raised from them are distinctly more variable than those from typical shrubs of the parental species, and they also show some segregation in the direction of their presumed parents. Under natural conditions, therefore, *C. thrysiflorus* and *C. dentatus* are distinct and have been so for probably hundreds of thousands of years, while occupying different ecological niches in the same geographic region. The creation of a new habitat by man has at least temporarily broken down the ecological isolation which previously separated them and has therefore reduced greatly their distinctness from each other.

One of the earliest described examples of ecological isolation is that of *Viola arvensis* and *V. tricolor* in Denmark (Clausen 1921, 1922). These two species have different chromosome numbers ($n = 17$ in *V. arvensis* and $n = 13$ in *V. tricolor*), but hybrids between them are fertile and segregate in the F_2 generation to give a great variety of recombination types. In nature, only a very small proportion of the possible recombinations is found in any abundance, and these approach two modes, one of them small-flowered, with yellowish-white petals and pinnate stipules (*V. arvensis*), and the other large-flowered, with blue or violet petals and palmate stipules (*V. tricolor*). The former is strongly calcicolous, while *V. tricolor* is found mainly on acid soils. Intermediate and recombination types can compete successfully only on neutral or faintly acid soil. Since chromosome numbers intermediate between 17 and 13 have not become established in this group, this cytological difference probably reinforces the ecological isolation in keeping the two species separate.

Another good example of ecological isolation is provided by two species of goldenrod of the eastern United States, *Solidago rugosa* and *S. sempervirens* (Goodwin 1937). The former is characteristic of dry, sterile fields over a large area, while *S. sempervirens* occurs in salt marshes along the entire coast. In general, they maintain themselves as distinct, but the hybrid between them, *S. asperula*, is not uncommon. In most localities, only solitary or few hybrid plants are found, but where the transition from a salt marsh to a neighboring field passes through a band of brackish marsh, rather extensive hybrid swarms may occur. Goodwin found that F_1 hybrids between *S. rugosa* and *S.*

sempervirens are fully fertile, but that seed from these plants germinates rather poorly and yields many weak F_2 individuals. In this example, therefore, the two species are kept apart both by ecological isolation and by hybrid sterility effective in the second generation.

The species of *Iris* growing in the Mississippi Delta afford an equally striking and well-analyzed example. The two most common ones are *I. fulta*, a slender, reddish-flowered species inhabiting the clay alluvial soil of natural levees in the shade of the swamp trees, and *I. hexagona* var. *giganticaerulea*, a stouter species with violet-purple flowers which is found in the mucky soil of alluvial marshes, usually growing in full sun (Viosca 1935). When these habitats are disturbed, through the cutting of trees in the habitat of *I. fulta* and the ditching and draining of the marshes occupied by *I. giganticaerulea*, opportunity is provided for hybridization on a large scale and the establishment of the hybrid derivatives in the new habitats formed by these disturbances (Viosca 1935, Riley 1938, Anderson 1949). In *Iris*, as in *Solidago* and *Ceanothus*, the evidence that ecological isolation is the principal barrier between the species concerned is strengthened by the fact that a disturbance of this barrier causes the appearance of large numbers of hybrids and hybrid derivatives, and thereby a great increase of gene flow between the two species.

Marsden-Jones and Turrill (1947) have described a good example of ecological isolation in the flora of Great Britain. *Silene maritima*, a plant of pebble beaches, sea bluffs, scree (talus), and rock faces, is distinguished by several easily recognizable characters from *S. vulgaris*, a common plant of fields and roadsides. When crossed artificially, the two species form vigorous, fertile hybrids in both the F_1 and later generations, so that internal isolating barriers between them are absent. Yet the authors have never observed intergradation or evidence of extensive hybridization between these two species in nature, apparently because their natural habitats are so different that they never have an opportunity to cross. This may be connected with the fact that *S. vulgaris* has entered Britain rather recently, perhaps only since the advent of agricultural man. Before *S. maritima* can be considered completely distinct and isolated from *S. vulgaris*, the absence of intergradation should also be demonstrated for their

populations on all parts of the European continent within the range of *S. maritima*. Other examples of sympatric species separated mainly by ecological isolation are *Geum urbanum* and *G. rivale* (Marsden-Jones 1930), *Silene cucubalus* and *S. alpina* (Turrill 1936b), and *Cistus monspeliensis* and *C. salviifolius* (Dansereau 1939).

Ecological isolation as an accessory mechanism, acting to reinforce the discontinuity produced by seasonal isolation or partial hybrid sterility, is a rather frequent phenomenon. Further examples of it will be given later in this chapter.

SEASONAL ISOLATION

Isolation of two populations because of different blooming seasons is occasionally found in plants. One of the best examples is *Lactuca canadensis* and *L. graminifolia* (Whitaker 1944). These two species, although rather different morphologically, have the same chromosome number ($n = 17$), and artificial hybrids between them are fully fertile. In the southeastern United States, where they occur together as common roadside weeds over a large area, they are ordinarily kept distinct through the fact that *L. graminifolia* blooms in the early spring and *L. canadensis* in the summer. They sometimes overlap slightly in their natural blooming periods, and under these conditions hybrid swarms can occasionally be developed.

An example of seasonal isolation which appears to be effective in one part of the range of a species but not in another is that of a Chinese species of the Composite, tribe Cichorieae, *Ixeris denticulata* (Stebbins 1937). In northern China are found two subspecies: *typica*, which blooms in the fall, and *sonchifolia*, which is a spring bloomer. In this region the two forms are amply distinct in external morphology, and one systematist has placed them in different genera. But in western and southwestern China there occurs another subspecies, subsp. *elegans*, which blooms in the summer. Judging from herbarium specimens, subsp. *elegans* is connected by a series of transitional forms to both *typica* and *sonchifolia*. Both *typica* and *sonchifolia* have the chromosome number $2n = 10$ (Babcock, Stebbins, and Jenkins 1937) and similar chromosome size and morphology. Although they have not been crossed, there is good reason to believe that the

hybrid between them would be vigorous and fertile, since *I. denticulata* subsp. *typica* forms fertile hybrids with the more distantly related *I. (Crepidiastrum) lanceolatum* (Ono and Sato 1935, 1937, 1941).

Seasonal isolation as a means of reinforcing ecological and geographic isolation is well illustrated in certain species of pines of the California coast. *Pinus radiata* and *P. muricata* are found together on the Monterey Peninsula, in the site previously described for *Ceanothus thyrsiflorus* and *C. dentatus*. The two species are amply distinct morphologically; *Pinus radiata* has needles in groups of three, while those of *P. muricata* are in two's, and the color and texture of the needles is very different. The cones of *P. radiata* are relatively large, asymmetrical, and with blunt cone scales, while those of *P. muricata* are smaller, more symmetrical, and with sharp pointed scales. At Monterey, *P. muricata* occupies the poorer sites of the ridge crests, and its stands are therefore completely surrounded by a dense forest of *P. radiata*, which grows in the better soils. Nevertheless, observations of Drs. Palmer Stockwell, H. L. Mason, and the writer indicate that intermediate trees, while not infrequent, form only a fraction of 1 percent of the stand and appear less vigorous and productive than typical *P. radiata* or *P. muricata*. This fact is probably due partly to the poorer adaptation of the intermediate gene combinations, but the possibility of hybridization is greatly reduced by the fact that in normal seasons in this area, *P. radiata* sheds its pollen early in February, while the pollen of *P. muricata* is not shed until April.

P. radiata is kept apart from another species of this group, *P. attenuata*, by a combination of geographical, ecological, and seasonal isolation. The distribution of *P. radiata* is strictly coastal and it occurs in three widely separated localities. That of *P. attenuata* is mostly in the middle and inner coast ranges and in the Sierra Nevada. But near Point Año Nuevo, in Santa Cruz County, *P. attenuata* grows near the coast and adjacent to *P. radiata*. In this region they appear to be ecologically isolated, since *P. radiata* grows only on the seaward side of the ridges, while *P. attenuata* is confined to their crests on the landward side, out of sight of the ocean. They never grow intermingled, but in one place the forest of *P. radiata* is immediately bordered by an open

stand of *P. attenuata*. In this area intermediates have been found, but, as in the previous examples, they form less than 1 percent of the population. Seasonal isolation also enters here. The pollen of *P. attenuata* is shed in April, and therefore much later than that of *P. radiata*, at least in this locality. Hybrids between these two species produced at the Institute of Forest Genetics, Placerville, have proved vigorous and fertile in both the F_1 and the F_2 generations (Stockwell and Righter 1946).

MECHANICAL ISOLATION

Among the three types of isolating mechanisms which act in the reproductive phase of the parental species, mechanical isolation probably plays the most important role. It is particularly effective in plants with elaborate floral structures adapted to insect pollination, like the milkweeds (Asclepiadaceae) and Orchidaceae. In the former group, the sticky pollen is contained in sacs or pollinia, which are attached in pairs by a clip. This structure is so designed that insects which visit the flowers of *Asclepias* for their nectar invariably pick up one or more of these pollinia on their legs. Pollination is completed by the insertion of the pollinia into slits in the stigma of the flower, which match in size and shape the pollinia of the same species. Nevertheless, as observed by Woodson (1941), the insertion of the pollen clips into the stigmatic slits is a difficult operation, and for this reason only a small proportion of the flowers of *Asclepias* ever become pollinated. This seemingly inefficient mechanism for cross-pollination is in one way very efficient. The various species of this genus differ from each other in the shape of both their pollinia and their stigmatic slits. These differences are large enough so that insertion of the pollinium of one species into the stigma of another is well-nigh impossible. The result is that in spite of the fact that several species of *Asclepias* exist side by side in the eastern United States and many other parts of the world, interspecific hybridization in nature is almost completely absent, and the species are very sharply set off from one another (Woodson 1941). Mechanical isolation, although reducing greatly the reproductive potential of individual plants, maintains the integrity and thereby the adaptive peak represented by the species. The selective disadvantage of a low percentage of fertilized flowers is probably less in

Asclepias than it is in many other genera. Each individual plant is very long-lived and produces large numbers of flowers each year; while the capsule which is formed whenever pollination is effective contains large numbers of seeds. These factors undoubtedly contribute toward the ability of *A. syriaca* and other species to become aggressive weeds, in spite of their apparently inefficient pollination mechanism.

The elaborate floral structures of the orchids probably serve a similar purpose. That the often bizarre and fantastic shapes of these flowers are adapted to securing cross-pollination by insects has long been recognized, and some of them are graphically described in the classic work of Darwin (1862). Although studies of this nature have unfortunately been largely neglected in recent years, one device for insect pollination unknown to Darwin and his contemporaries has been explored. This is the phenomenon of pseudocopulation, in which resemblance of the orchid flower to a female insect attracts the male of that species and causes him to attempt his normal biological function. During this attempt, the insect delivers pollen to the stigma of the orchid flower and receives pollinia from it. According to the excellent review of this subject by Ames (1937), the first example known of pseudocopulation is that of the Australian species, *Cryptostylis leptochilla*, which lures the fly *Lissopimpla semipunctata*. Still more interesting and instructive are the examples described by Pouyanne of various species of the European and North African genus *Orphrys*, which are pollinated by male bees belonging to the genera *Andrena* and *Scolia*. The differences between the flowers of these species of *Orphrys* consist partly of superficial resemblances to the females of different species of bees, so that each orchid species attracts only one species of bee. Furthermore, the resemblance is secured in entirely different ways by different species of orchids. For instance, in *Orphrys fuscata* and *O. lutea*, which attracts species of *Andrena*, the "abdomen" of the imitation female is oriented so that the male alights with his posterior end adjacent to the stigmatic column, and so receives and delivers the pollen on his abdomen; while *Orphrys speculum* attracts the male of *Scolia ciliata* in such a way that he receives and delivers the pollen with his head (Figure 22).

Pseudocopulation may be a more widespread phenomenon

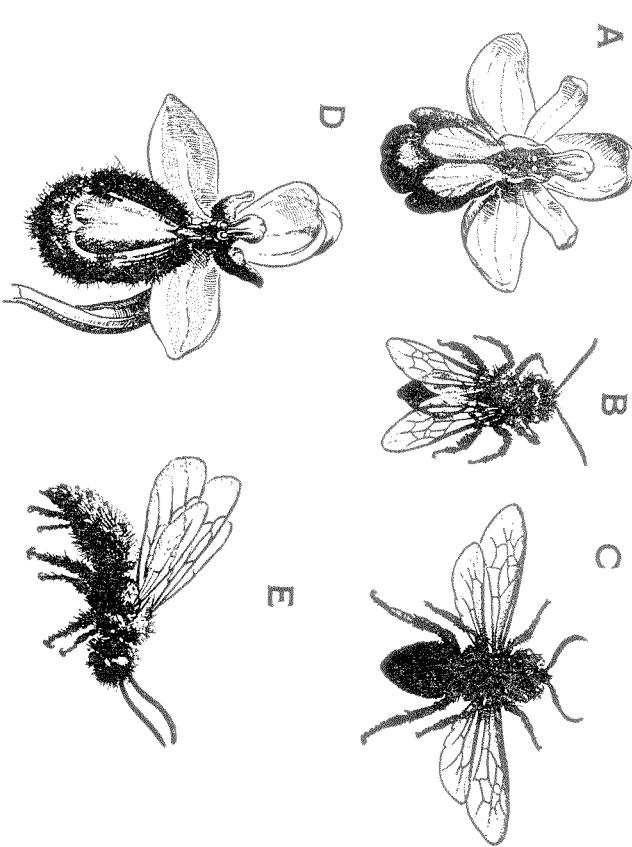


FIG. 22. A, a single flower of *Ophrys fusca*; B and C, males of two species of bees (*Andrena nigroaenea* and *A. timmerana*) which pollinate it; D, a flower of *Ophrys speculum*; and E, a male bee of the species (*Scolia ciliata*) which pollinates it. From Ames 1937.

than our present knowledge indicates. Dr. W. H. Camp has told the writer of observing the pollination of orchid species in the South American tropics by the males of Syrphid flies. As a method of securing pollination of individual flowers it may not be any more efficient than the more familiar and widespread one of attracting female insects in search of pollen or nectar. But the specificity of this device obviously helps greatly to prevent accidental cross-pollination between different species, and therefore to strengthen reproductive isolation.

In the orchids even more than in the asclepiads the nature of their pollination mechanisms seems to be well adjusted to their mode of life as a whole and to be partly responsible for the pattern of variation and evolution found in the family. Orchids are very long-lived as individual plants, so that failure of seed formation in any one year is no great detriment to them. Further-

more, the number of seeds produced in a single capsule is perhaps the largest in the entire plant kingdom, so that the number of successful pollinations required is less than in most other families. In the temperate regions, with their climatic vicissitudes and comparative poverty of insect life, this system has been only a partial success, and orchids are for the most part rare and comparatively poor in species. But the moist tropics, with their optimum climatic conditions and richness of insect fauna, have proved to be such a favorable environment for the development of these mechanisms for cross-pollination and for reproductive isolation between species that in most of the moister parts of the tropics the orchids lead all other families in the number of their species, and often also in number of individuals.

Recent observations of Mather (1947) have shown that in flowers with an elaborate and distinctive structure and color mechanical isolation can be achieved between two populations even though they are pollinated by the same species of insect. Cultures of two different species of the genus *Antirrhinum*, sect. *Antirrhinastrum*, *A. majus* and *A. glutinosum*, were grown in alternating blocks of rows, so as to ensure the maximum chance for cross-pollination. The species have long been known to be cross-compatible and *A. glutinosum* is self-incompatible, so that artificial hybrids are obtained with the greatest of ease (Baur 1924, 1932). Nevertheless, in seed harvested from these plots only a very small percentage of hybrids was secured. Following the course of individual bees, Mather found that in any particular flight they nearly always confine their visits to the flowers of one species. After pollinating the flowers of several plants of the species first visited, they not infrequently fly directly over several rows of other species to reach another plant of the one on which they began their rounds.

PREVENTION OF FERTILIZATION

The two other barriers which act in the reproductive phase of the parental species, namely, failure or ineffectiveness of pollen tube growth and failure of fertilization, are rarely the primary cause of reproductive isolation between closely related species. Kostoff (1943, pp. 704-706) lists 68 interspecific combinations in the genus *Nicotiana* which failed because the pollen tubes were

unable to reach the ovules. But 50 of these were between species having different chromosome numbers, and the hybrids, had they been obtained, would probably have been weak, sterile, or both.

Of the remainder, all but one were intergeneric, and similar hybrids in which F_1 plants were obtained always proved to be completely sterile. The cause for this failure of pollen tube growth is often the fact that the style of the maternal species is much longer than that of the species which contributes the pollen, so that the pollen grains are not adapted to traversing the long distance from the stigma to the ovary. In addition, the relatively large, thick pollen tubes produced by some polyploid species have difficulty in penetrating the smaller styles of diploids, so that in some combinations between diploids and tetraploids fertilization fails when the tetraploid is the pollen parent, but succeeds when it is the maternal one.

In the genus *Datura*, Buchholz, Williams, and Blakeslee (1935) found that pollen tubes grow faster in styles belonging to their own species than in foreign ones, and that they often burst in the styles of foreign species. In this genus, however, the species are still more strongly isolated by barriers of hybrid inviability, so that even though pollen tube growth and fertilization are successful, hybrids are rarely obtained (see below). Similarly, *Zea mays* and various species of *Tripsacum* are isolated from each other by the inability of the *Zea* pollen grains and male gametes to function in the ovules of *Tripsacum* and the barrier to the reciprocal hybrid caused by the great length of the styles, or silks, in maize (Mangelsdorf and Reeves 1939). Mangelsdorf secured hybrids between the two genera by artificially reducing the style length of *Zea*, but the high degree of pollen and seed sterility in these F_1 hybrids is perhaps a more potent barrier to gene interchange between these two species than is their initial incompatibility. Difference between the osmotic tension of pollen grains and style tissues is often a barrier to hybridization between species having different chromosome numbers (Watkins 1932, Boyes and Thompson 1937, Schlosser 1936), but in these examples sterility of F_1 hybrids serves as an additional and perhaps a more significant barrier of reproductive isolation between the species concerned.

HYBRID INVIAILITY OR WEAKNESS

The barriers which act by preventing the growth or reproduction of F_1 hybrids are probably the most fundamental and certainly the most widespread of those which separate plant species. They may become manifest at any stage from the beginning of the growth of the hybrid zygote to the maturity of the segregating genotypes of the F_2 generation. The term hybrid inviability may be applied to the inability of the hybrid zygote to grow into a normal embryo under the usual conditions of seed development. It may be caused by disharmony either between the parental sets of chromosomes or between the developing embryo and the endosperm which surrounds it. If the former is the case, then normal mature hybrids cannot be secured under any conditions.

If the growth of the embryo is inhibited by the endosperm, vigorous embryos can often be obtained by dissecting the young embryo from the seed and growing it artificially in a nutrient solution. One of the earliest successful experiments of this nature was that of Laibach (1925) on *Linum perenne* \times *austriacum* and its reciprocal. When *L. perenne* is the maternal parent, the embryo reaches maturity, but cannot sprout from the seed. If dissected out artificially, it grows into a vigorous and fertile F_1 hybrid. In the reciprocal cross, *L. austriacum* \times *perenne*, hybrids can be obtained by dissecting the very young embryos out of the developing seed and growing them in a nutrient solution. Similar success was obtained by this means in crosses between peach varieties by Blake (1939) and Lammerts (1942), in *Secale cereale* \times *Hordeum jubatum* by Brink, Cooper, and Ausberman (1944), and particularly in hybrids of the genus *Datura* by Blakeslee, Satina, and their associates (Blakeslee and Satina 1944, Blakeslee 1945, McLean 1946).

What may be another way of overcoming a barrier of hybrid inviability is described by Pisarev and Vinogradova (1944) in crosses between *Triticum* and *Elymus*. Young embryos of *Triticum vulgare* were dissected out and grafted onto the endosperm in ovules of *Elymus arenarius*. There they finished their growth and germinated from *Elymus* seeds. The mature plants obtained by this method were typical of *Triticum* in appearance, but their reaction to hybridization with *Elymus* differed in that they could

be used successfully as maternal parents in the cross *Triticum vulgare* \times *Elymus arenarius*, while *Triticum* plants of the same variety grown from normal seed could not function as the pollen or as the female parent in the same hybrid combination. This remarkable result should be repeated with this or other material.

As yet very little is known of the physiological or developmental nature of hybrid inviability, and its genetic basis has been discussed in only a few instances. A well-known example is the interspecific lethal gene found by Hollingshead (1930a) in *Crepis tectorum*, which has no effect on its own species, but causes the early death of the hybrid seedlings in crosses with *C. capillaris*, *C. leontodontoides*, and *C. bursifolia*. This gene, however, is not the principal agent for isolating the species concerned. The viable hybrids produced when the *C. tectorum* parent does not carry this gene are in every case highly sterile, due to lack of pairing and irregularity of chromosome behavior at meiosis. In the genus *Hutchinsia*, Melchers (1939) has found a similar gene isolating a certain strain of *H. alpina* from other races of this species and from *H. brevicaulis*. In this case the vigorous hybrid derived from strains of subsp. *tyifica* not carrying the lethal gene is fertile, so that the establishment of this gene in all the individuals of any isolated subpopulation of *H. alpina* would transform such a population into an incipient new species. Similar genes have been found by Brieger (1944a) in *Nicoliana longiflora* \times *sanderae*.

In some instances, the hybrid embryo may produce a plant which is too weak to reach maturity or flower normally. A spectacular type of hybrid weakness due to genic disharmony is found in certain hybrids of the genus *Nicotiana*, which form tumors in their vegetative parts (Kostoff 1930, 1943, pp. 613–16, Whitaker 1934b).

In the genus *Epilobium* the extensive studies of Michaelis (1931, 1933, 1938, 1940, Michaelis and Wertz 1935), Lehmann (1931, 1936, 1939, 1942, 1944), and others have revealed the presence of barriers of hybrid weakness and sterility which result from the interaction of certain hybrid genotypes with the plasma of one of the parental species. For instance, the F₁ hybrid *E. luteum* \times *hirsutum* is vigorous and fertile when *E. luteum* is the maternal plant, but in the reciprocal cross, in the plasma of *E.*

hirsutum, is weak and sterile. Michaelis, by pollinating the vigorous *E. luteum* \times *hirsutum* F₁ with *E. hirsutum* pollen and continuing this type of backcross for 13 generations, obtained a plant which resembled *E. hirsutum* in all of its external characteristics, but had certain cytoplasmic characteristics of *E. luteum*. It formed vigorous F₁ hybrids when pollinated with *E. luteum*, and its cytoplasm resembled that of the latter species in its relatively high permeability to electrolytes and its susceptibility to sudden changes in temperature. Both Michaelis and Lehmann demonstrated numerous genetic differences between different strains of *E. hirsutum*, as well as the related *E. roseum* and *E. montanum*, in their reaction to *E. luteum* and other species. That these differences are genetically controlled is shown by the fact that, for instance (Lehmann 1939), an F₁ hybrid between two strains of *E. hirsutum*, when outcrossed to *E. adenocaulon*, gives two types of interspecific hybrids in a 1:1 ratio. Furthermore, some races of *E. hirsutum* give inhibitions of growth when crossed with other races of the same species (Lehmann 1942, Brücher 1943).

The explanation of these results is not clear. That, in contrast to the majority of plant species which have been compared by means of hybridization, the species of *Epilobium* differ markedly in their cytoplasm, is certain. A similar phenomenon appears with respect to the plastids in the genus *Oenothera* (Renner 1936), which likewise belongs to the family Onagraceae. Cytoplasmic and plastid differences of such a striking nature may be a peculiarity of this and some other families of seed plants. The constancy of the plasma and its apparent partial independence of the genotype in *Epilobium* are as yet unique among known examples in the higher plants, although similar phenomena have been found by Wettstein (1937) in hybridizations between species and genera of mosses. Here, however, the reciprocal differences are not inhibitions of growth, but alterations in leaf shape, capsule shape, and other characteristics. There are some other examples of reciprocal differences in hybrids between plant species, but none of these have been investigated with anything like the thoroughness of those mentioned above. Good reviews of this subject are those of Correns and Wettstein (1937) and Caspari (1948).

HYBRID STERILITY

In his thorough review of the subject of hybrid sterility, Dobzhansky (1941) has shown clearly that the failure of hybrids to produce offspring is not connected with any constitutional weakness of the organism as a whole, but rather with specific disharmonies between the parental gene complexes which act during the development of the gonads, at the time of meiosis, or during the later development of the gametophytes or gametes. The earliest classifications of the phenomena of hybrid sterility were based on the time when the sterility becomes manifest. Thus, Renner (1929) distinguishes between gametic and zygotic sterility. The former is the degeneration of the spore tetrads and gametophytes between the time of meiosis and antesis, and is recognized by the abortion of pollen or ovules, while the latter is the failure of the zygote to develop after fertilization, and is manifest through the abortion of seeds even though the ovules and pollen are normal. Münzing (1930a) distinguishes between haplontic sterility, which is the gametic sterility of Renner, and diplontic sterility, which may act either in the diploid tissue of the F_1 hybrid before meiosis or in the zygotes of its offspring. Both of these classifications are useful for certain purposes, but the separation of hybrid sterility phenomena into two types, genic sterility and chromosomal sterility (Dobzhansky 1933, 1941, p. 292) is more fundamental. Genic sterility includes all types which are produced by failure of the sex organs to develop up to the point where meiosis can take place or by genetically controlled abnormalities of the meiotic process itself, such as abnormalities of spindle formation and genetically controlled asynapsis or desynapsis (Clark 1940, Beadle 1930, Li, Pao, and Li 1945, etc., see review in Stebbins 1945). Chromosomal sterility, on the other hand, results from lack of homology between the chromosomes of the parents of a hybrid. When the parental species differ radically from each other in chromosome number, as in the classic example of *Drosera longifolia* \times *roundifolia* (Rosenberg 1909), or when these parents are distantly related to each other, as in the cases of *Nicotiana sylvestris* \times *tomentosiformis* ("Rusbyi," Goodspeed and Clausen 1928), *Raphanus sativus* \times *Brassica oleracea* (Karpenko 1927, 1928), and *Gossypium arboreum* \times *thurberianum* (Skovsted 1934, Beasley 1942), chromosomal sterility is manifest.

The classic example of this less pronounced type of chromosomal sterility is *Primula veris* \times *floribunda*. Both parental species here have the haploid chromosome number $n = 9$, and although the F_1 hybrid is completely sterile, its chromosomes usually form at meiosis nine loosely associated bivalents (Newton and Pellew 1929, Upcott 1939). Without further data, one would suspect this to be an example of genic sterility, induced by some type of complementary factor system. But when the somatic chromosome number of this F_1 hybrid is doubled, the resulting allopolyploid, *P. keuensis*, is fully fertile and forms mostly bivalents at meiosis. Although differences in the segregation ratios of genes in a tetraploid as compared to a diploid could account for a considerable difference in their fertility on the basis of the action of complementary gene factors, it could not account for the change from complete sterility to full fertility. The explanation of this change, as suggested by Darlington (1937, pp. 198–199) and Dobzhansky (1941, p. 328), is best made as follows. If we assume that the chromosomes of *P. floribunda* resemble those of *P. veris* in respect to their centromeres and at least one of their ends, then they can pair and form chiasmata in an apparently

normal fashion. But if differences exist, in the form of small, nonhomologous segments, then this apparently normal pairing actually involves different kinds of chromosomes, and the reduction in chiasma frequency which is found in *P. verticillata* \times *floribunda* and similar hybrids is due to the inability of certain segments of their chromosomes to pair and to form chiasmata. The gametes produced by such hybrids are inviable, not because they have deviating chromosome numbers, but because they contain unbalanced, disharmonious combinations of chromosomal material. The production of an allopolyploid by somatic doubling of the chromosome number should in this case result in cells with two sets of exactly similar chromosomes. Pairing and separation of chromosomes in sporocytes consisting of such doubled cells would produce gametes which, in both the number and the genetic content of their chromosomes, would correspond to the somatic cells of the F_1 hybrid and would therefore be perfectly viable and functional. Furthermore, since each chromosome would find in the sporocytes of the doubled tissue a mate exactly like itself, it would pair only occasionally with the partially homologous chromosome derived from the opposite species. In the terminology of Darlington (1937, pp. 108-9), the differential affinity between exactly similar as compared to partly homologous chromosomes would lead to preferential pairing between the chromosomes derived from the same parental species. This explains the fact that although the diploid F_1 hybrid *P. verticillata* \times *floribunda* usually forms nine bivalents, the allotetraploid *P. kevensis* forms only 0 to 3 quadrivalents.

The situation can be represented diagrammatically as follows, where F represents any chromosome of *P. floribunda*, V a chromosome of *P. verticillata*, while V_F and F_V represent chromosomes containing mixtures of parental chromosomal material.

Meiosis in parents:

$$\frac{F}{F} \text{ and } \frac{V}{V}$$

Constitution of parental gametes:

$$F \text{ and } V$$

Constitution of F_1 somatic cells:

$$FV$$

Pairing at meiosis in F_1 :

$$\frac{F}{V}, \text{ with crossing over}$$

Constitution of F_1 gametes:

F_v and V_F , all or nearly all inviable

Constitution of allopolyploid somatic cells:

$$FFVV$$

Pairing at meiosis in allopolyploid:

$$\frac{F}{F}, \frac{V}{V}$$

Gametes of allopolyploid:

all FV and viable.

It is thus evident that, as Dobzhansky has pointed out, many examples of chromosomal sterility can be detected only by the chromosome behavior and fertility of the allopolyploid derived from the F_1 hybrid. The possibility that sterility in hybrids with apparently normal meiosis might be caused by structural hybridity involving small chromosomal segments was first suggested by Sax (1933) for *Campsis chinensis* \times *radicans*. Müntzing (1938) adopted this explanation for the partial sterility of *Guleopsis tetrahit* \times *bifida*, as well as for a similar phenomenon in crosses between certain races of *G. tetrahit*. In the latter examples, he obtained strong circumstantial evidence in favor of his hypothesis. Certain interracing F_1 hybrids within *G. tetrahit* form 50 percent of inviable pollen in spite of the presence of 16 pairs of chromosomes at meiosis. In other combinations, the same degree of sterility is accompanied by the occasional presence of two univalent at meiosis, while still other 50 percent sterile interracing F_1 hybrids form at meiosis 14 bivalents and a chain or ring of four chromosomes. This latter configuration is, of course, strong evidence of heterozygosity for interchange of a chromosomal segment. The similar nature of all of the partly sterile interracing hybrids in *G. tetrahit* suggests that their sterility is due to a common cause, structural hybridity, which may sometimes become manifest in the nature of the chromosome pairing, but most often does not.

The widespread occurrence in the higher plants of chromosomal sterility due to heterozygosity for small structural differences is evident from the fact that a large proportion of hybrids between closely related species having the same chromosome number exhibit apparently normal chromosome behavior at meiosis in spite of their partial or complete sterility. Such hybrids have been reported in *Apocynum* (Anderson 1936a), in *Bromus* (Stebbins and Tobgy 1944), in *Ceanothus* (McMinn 1942, 1944),

in *Collomia* (Hiorth 1934), in *Crepis* (Babcock and Emsweller 1936, Jenkins 1939), in *Festuca elatior* \times *Lolium perenne* (Peto 1933), in *Ixeris*, subg. *Crepidiasstrum* \times subg. *Parixeris* (Ono and Sato 1935, Ono 1937, 1941), in *Lactuca* (Whitaker and Thompson 1941), in *Layia* (Clausen, Keck, and Hiesey 1941), in *Lycopersicum* (Lesley and Lesley 1943, MacArthur and Chiasson 1947), in *Melica* (Joranson 1944), in *Papaver* (Fabergé 1944), in *Phaeolus* (Lamprecht 1941), in *Polygonatum* (Suomalainen 1941), in *Populus* (Peto 1938), in *Setaria* (Li, Li, and Pao 1945), in *Solanum* (Propach 1940, Tatebe 1936), in *Taraxacum* (Pod-dubnaja-Arnoldi 1939), in *Tradescantia* (Anderson and Sax 1936), in *Tragopogon* (Winge 1938), in *Verbena* (Dermer 1936, Schnack and Covas 1945a,b), in *Elymus* \times *Sitanion* (Stebbins, Valencia, and Valencia 1946a), and probably in others. In *Lycopersicum esculentum* \times *peruvianum* and some of the *Solanum* hybrids evidence from allopolyploids has shown that their sterility is genic and is retained in the polyploid, while those of *Bromus* (Stebbins 1949b), *Melica*, *Tradescantia* (Skirm 1942), and *Elymus* \times *Sitanion* recovered their fertility upon doubling, as did *Primula kewensis*. Present evidence suggests, therefore, that in the great majority of hybrids between closely related species the decision as to whether the sterility is genic or chromosomal, or both can be made only after allopolyploids have been obtained. The apparently widespread existence of chromosomal sterility due to heterozygosity for structural differences so small as not to materially influence chromosome pairing at meiosis has led the writer to propose a name for this situation: *cryptic structural hybridity* (Stebbins 1945; Stebbins, Valencia, and Valencia 1946a).

The fact must be emphasized here that much of this cryptic structural hybridity is difficult to discover only because in most plants the best stage for studying the gene-by-gene pairing of the chromosomes, the pachytene stage of meiosis, is not clear enough for analysis. In nearly all interspecific hybrids, chromosome pairing is analyzed at the first meiotic metaphase, when the chromosomes are strongly contracted and are associated at only a few points along their length. Even rather large structural differences between partly homologous chromosomes could not be detected at this stage.

The first suggestion as to how cryptic structural hybridity could originate was made by Sturtevant (1938). He pointed out that in some cases an inverted chromosome segment may undergo reversion, so that for most of the gene loci the original position is restored. But the chances are very small that the breaks which produce the reversion will occur at exactly the same point as those which produced the original inversion. A chromosome which has suffered inversion and reversion will therefore differ from its original ancestor by possessing two small segments in an entirely different position. For instance, if the loci on the original chromosomes are ABCDEFGHI, then the original inversion will give the arrangement ABGFEDCHI, and reversion will very likely produce something like ABHCDDEFGI. Now, if the inverted chromosome is associated in a hybrid with the original one, it will form an apparently normal bivalent, since the displacement of the H segment will be manifest only by two small foldbacks at pachytene, similar to those formed by heterozygosity for small deletions, or it may be completely obscured by nonhomologous pairing. But if chiasma formation and crossing over occurs in any of the regions C to G, two abnormal types of chromosomes will be formed, namely ABHCDEFHI and ABCDEFGI, the first containing a duplication and the second a deficiency. Such gametes are likely to be inviable or less viable than normal ones, so that sterility in a hybrid heterozygous for one such inversion-reversion combination will be one half the percent of frequency of chiasmata in the originally inverted segment.

Systems of translocations can occur which will have the same effect as Sturtevant's example, but will produce even more pronounced sterility. If a translocated segment becomes restored to its original chromosome by a second translocation at a slightly different point, pairing between the retranslocated chromosomes and the unaltered ones will be apparently normal, but independent segregation of the slightly unequal bivalents thus formed will automatically lead to the possession by 50 percent of the resulting gametes of a duplication and a deficiency, and therefore of nearly or quite 50 percent sterility of the hybrid. A similar situation, which might arise with even greater frequency in nature, would be produced by pairing between two chromosome sets, both of which were descended from the same original an-

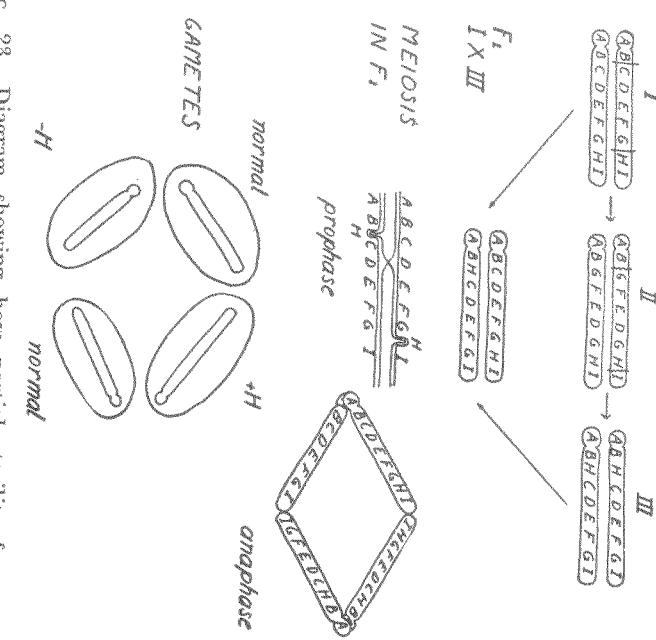


FIG. 23. Diagram showing how partial sterility from cryptic structural hybridity could be produced by means of inversion and reinversion. Further explanation in the text. Original.

cestor and had undergone translocations involving the same two chromosome pairs at slightly different loci. Diagrams of these situations are given in Figs. 23 and 24. It is evident that duplication-deficiency gametes and consequent partial sterility result in this example, not from abnormal pairing within individual bivalents, but from independent segregation of different bivalents. In such hybrids, chromosome pairing even at its most intimate stage, the pachytene of mid-prophase, might appear perfectly normal, since nonhomologous pairing (McClintock 1933) could occur in the arms bearing the translocations.

The hypothetical examples just cited are undoubtedly vastly more simple than most of the conditions of cryptic structural hybridity existing in nature. Müntzing's data (1930a) on the segregation of fertility in the F₂ generation of *Galeopsis tetrahit* × *bifida* indicate that partial sterility in this hybrid is due to a large number of factor differences, each of which may represent a small translocated, inverted, or otherwise displaced chromosome seg-

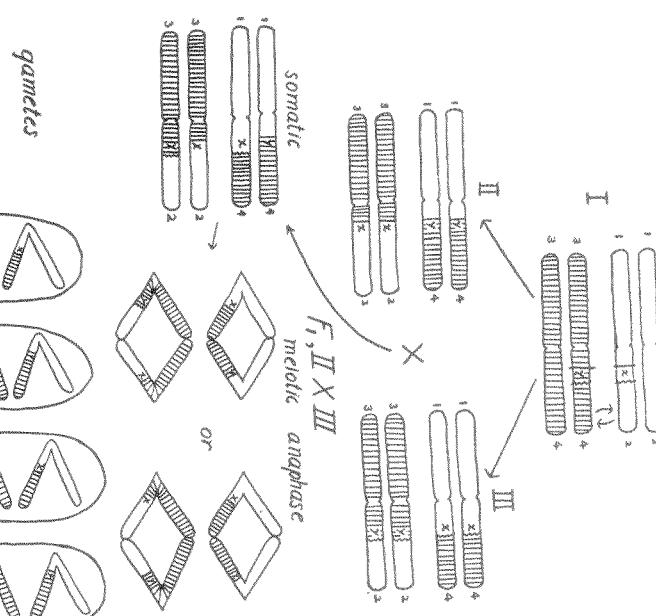


FIG. 24. Diagram showing how partial sterility from cryptic structural hybridity could be produced by means of two independent reciprocal translocations involving the same two pairs of chromosomes. Further explanation in the text. Original.

ment. Similar results have been obtained by Stebbins (1949b) in progeny of hybrids within the complex of *Bromus carinatus* and that of *B. catharticus*. Stebbins, Valencia, and Valencia (1946a) have considered the number of small chromosomal segments for which an F₁ hybrid may be heterozygous before marked chromosomal disturbances will be evident at meiosis. Based on the accurate data of Stadler, McClintock, and others on the sizes of gamete lethal and semilethal deficiencies in maize, they computed that two chromosomes may differ from each other by as many as five or six segments which are large enough to produce lethality or semilethality of the gametes when absent from the gametic complement; and still these two chromosomes would

have enough homologous segments in common so that they could pair and form chiasmata with considerable regularity. In most diploid plant hybrids, therefore, fairly regular pairing could take place even though the chromosomal complements of the parents differed from each other by as many as 30 to 50 translocated or inverted segments. And as Sax (1933) has pointed out, heterozygosity for even four or five such segments could be expected to produce a high degree of gametic or haplontic sterility. The frequent presence of good chromosome pairing and at the same time of hybrid sterility of the chromosomal type is therefore to be expected to occur often in hybrids between closely related species.

There is no doubt that many examples of hybrid sterility include a combination of both genic and chromosomal sterility factors. This was first clearly demonstrated by Greenleaf (1941) for the hybrid *Nicotiana sylvestris* \times *tomentosiformis*. The diploid F_1 has little or no chromosome pairing and is completely sterile, as might be expected from the fact that its parents belong to different sections of the genus. When the chromosome number of this hybrid was doubled by means of regenerating callus tissue, the resulting allopolyploid had good chromosomal pairing and a high percentage of normal, viable pollen, but set no seed, either with its own or with any other pollen. This was due to the abortion of the embryo sacs at an early age, a condition apparently controlled by certain genetic factors or factor combinations. An example of a different type is *Aegilops umbellulata* \times *Haynaldia villosa* (Sears 1941b). Lack of pairing in the diploid F_1 of this intergeneric hybrid is undoubtedly due in large part to dissimilarity between the parental chromosomes and should therefore be termed chromosomal. But the allopolyploid produced from this hybrid remained sterile in respect both to pollen and to seed and showed a high degree of asynapsis, in spite of the fact that each chromosome had a mate with which it was completely homologous. Superimposed on the chromosomal sterility, this hybrid must have had a gene combination causing asynapsis or desynapsis, similar to those which arise as occasional mutations in good species. In the progeny of the allopolyploids between *Triticum aestivum*, on the one hand, and *Agropyron elongatum*, *A. trichophorum*, or *A. glaucum* on the other, Love and Suneson (1945) found more asynapsis than would be expected on the basis of lack

of chromosome homology alone, and Pope (1947) has found a considerable degree of asynapsis in the colchicine-induced allotetraploid of *Triticum aestivum* \times *Agropyron elongatum*. Similarly, Stebbins, Valencia, and Valencia (1946b) found genic sterility superimposed upon chromosomal sterility in *A. trachycaulum* ("*A. pauciflorum*") \times *Hordeum nodosum*, and Walters (unpublished) found the same in *Agropyron parishii* \times *Sitanion jubarum*.

DEGENERATION OF HYBRID PROGENY

The final barrier to gene interchange between species is effective only after the F_2 progeny have been produced. Clausen, Keck, and Hiesey (1940) reported that in *Zauschneria cana* \times *septentrionalis* the F_1 plants are vigorous and highly fertile as to seed setting, but the F_2 population consists largely of weak, disease-susceptible individuals, so that intermediates between the two species cannot be maintained. The same is true of *Layia gaillardiooides* \times *hieracoides* (Clausen, Keck, and Hiesey 1941) and was found by Goodwin (1937a) in *Solidago rugosa* \times *semperfervens*, Propach (1937) in *Solanum henryi* \times *verrucosum*, Syrach-Larsen (1937) in *Larix Gmelini* \times *kaempferi*, and Johnson (1947) in *Populus alba* \times *grandidentata*. The F_1 hybrid between *Gossypium arboreum* and *G. herbaceum* is likewise vigorous and fertile (Webber 1935, Hutchinson 1940, Sitow 1944) and frequently appears in fields in which its parent species are grown. But F_2 progeny are rarely found; they are apparently too weak to survive. Similarly, the writer has been informed by Dr. S. G. Stephens (oral communication) that while the hybrid between the two New World tetraploid cottons, *G. barbadense* and *G. hirsutum*, is highly fertile, its progeny are so weak that great difficulty is experienced in transferring a gene artificially from one species to the other.

The causes for this degeneration of hybrid segregates may be either genic or chromosomal. It is obvious that if two species differ widely in the physiology of their developmental processes, many recombinations of the various genetic factors affecting these processes will be disharmonious and will produce individuals not adapted to any environment. On the other hand, if chromosomal sterility is operating in the F_1 , micro- or megaspor-

can be produced which are capable of developing into gamophytes and functional gametes in spite of minute duplications or deficiencies, but the accumulation of two or more deficiencies in the zygote may well be fatal to it. Furthermore, McClintock (1942 and oral communication) has shown that different types of tissues have different degrees of sensitivity to the same deficiency. One which can be carried through the pollen grain and pollen tube may be completely lethal when homozygous in the embryo or endosperm. There is obviously no way of distinguishing between genic and chromosomal sterility or invisibility in F_2 segregates, and from the practical point of view the distinction is not very important. The highly significant fact about the examples cited in this section is that genetic barriers between species may exist even when they can cross easily and form vigorous, fertile F_1 hybrids. When this is the case, genetic criteria for the separation of species must be explored through the F_2 generation before the existence of isolating mechanisms can be regarded as proved or disproved.

THE INTERRELATIONSHIPS BETWEEN ISOLATING MECHANISMS AND INTERSPECIFIC DIFFERENCES

In the preceding sections reference has often been made to species separated not by one but by combinations of different isolating mechanisms. Too much emphasis cannot be given to such examples; they probably represent the normal condition in nature. Any one of the various isolating mechanisms may be present to a greater or lesser degree as a partial barrier between individuals or races belonging to the same species, and there are relatively few species in the plant or animal kingdoms which are separated by only one type of isolating mechanism. Isolation barriers are usually built up of many different, independent parts. If they are not complete, then the segregating progeny of interspecific hybrids inherit various elements of the original interspecific barrier. They may thus cause its temporary breakdown, or they may build up new isolating barriers upon the foundations of the old one and thereby become the ancestors of new species. This topic will be considered in the following chapter. Here we wish only to emphasize the point that the nature of interspecific isolation barriers precludes the possibility that species usually

arise full-fledged at a single step, barring the "cataclysmic" event of polyploidy. The isolating mechanisms responsible for the discontinuity between species, like the morphological and physiological differences between them, are compounded of numerous genetically distinct elements, which in most cases must have arisen and become established in the species populations over a considerable period of time.

Furthermore, the correlation between degree of similarity in morphological characteristics and the effectiveness of the isolation barrier between two species is by no means absolute. To be sure, closely similar species are most often separated by relatively weak systems of isolating mechanisms and can as a rule be hybridized more easily than widely divergent ones. But every systematist who has studied several different, unrelated groups of animals or plants is familiar with the fact that in some groups types which are widely divergent morphologically grade into each other through series of intermediate forms, while in other groups sharp distinctions can be drawn between species which in their external appearance are very similar. Species in unrelated groups are not equivalent to each other either morphologically or ecologically. A few examples may be cited to bring out this fact. In Chapter II the fact was mentioned that in the genus *Aquilegia* almost any two species can be hybridized and will give vigorous and fertile progeny in the F_1 and F_2 generations. This lack of genetic isolation extends even to the genus *Paraciquidgia*, which lacks the spurred petals characteristic of the columbines and therefore in some respects resembles the genera *Isopyrum* and *Anemoneella* as much as *Aquilegia*. Earlier in this chapter evidence was cited of extensive intergradation between species of *Ceanothus* which in their typical forms are widely divergent both morphologically and ecologically, and similar examples could be cited from many other genera, such as *Rhododendron* (Sax 1930), *Vaccinium* (Darrow and Camp 1945), *Vernonia* (Steyernark 1939), *Alseuosmia*, *Coprosma*, and *Veronica* ("Hebe") (Allan 1940). On the other hand, the examples of *Crepis neglecta-fuliginea* and the *Hemizonia virginata* complex, cited earlier in this chapter, are groups in which isolating mechanisms have developed extensively, accompanied by relatively little divergence in morphological characteristics and ecological preferences.

Further evidence of the relative independence of morphological divergence and the development of hybrid sterility is provided by analyses of the progeny of partly sterile interspecific hybrids. If the factors producing the visible differences between species are the same as or are genetically linked to those responsible for hybrid sterility, then we should expect to find that the relatively fertile F_2 segregates from a partly sterile F_1 would resemble morphologically one or the other of its parents, while those remaining intermediate, or possessing new recombinations of the parental characteristics, should be as sterile as the F_1 or more so. Five examples may be cited which bear on this point.

The first is the ingenious and illuminating study made by Anderson (1936a) and his colleague, R. E. Woodson, on the progeny of the partly sterile hybrid *Apocynum medium* (*A. androsaemifolium* \times *cannabinum*). In this study, the geneticist (Anderson) raised the segregating progeny from the hybrid together with that from both parental species and sent specimens of these plants, without information on their origin, to the systematist (Woodson) for identification. The interesting result was that some of the F_2 hybrid derivatives were near enough to one or the other parental species so that they were identified as aberrant forms of them, while others resembled their F_1 hybrid parent so much that they were called *A. medium*. The F_2 plants also varied in fertility, but there was apparently no close correlation between the morphological appearance and the fertility of the plants. Among those considered to be still intermediate (*A. medium*), some were highly fertile, though others were more sterile than the F_1 ; while those judged to be conspecific with either *A. androsaemifolium* or *A. cannabinum*, though mostly rather fertile, included the most sterile plants of all.

The second example, *Galeopsis tetrahit* \times *bifida*, has already been mentioned (Münzing 1930a). Münzing analyzed 355 F_2 plants of this cross, scoring them in respect to percentage of apparently normal pollen and to the number of characters in which they resembled either *G. tetrahit* or *G. bifida*. The result is shown in Table 5. Individuals with the value +5 resemble *G. tetrahit* in five out of the seven characters scored; those with the value 0 are exactly intermediate; and those with -5 resemble *G. bifida* in five out of seven characteristics. This table shows clearly the complete lack of

correlation between sterility or fertility and resemblance to one or the other of the original parents. The visible characters which differentiate the species and the sterility factors which partly isolate them segregate quite independently of each other.

TABLE 5
RELATION BETWEEN POLLEN FERTILITY AND MORPHOLOGICAL CHARACTERS IN *Galeopsis tetrahit* \times *bifida*, F_2 ^a

Value figure	Number of plants	Mean pollen fertility (percent)
-5	2	55-80 (range)
-4	17	71.90
-3	33	68.70
-2	49	67.30
-1	64	68.30
0	66	64.90
+1	61	67.40
+2	38	65.25
+3	17	60.75
+4	5	64.50
+5	3	35-75 (range)

^a Data from Münzing 1930a.

The third example is the thorough and careful analysis by Terao and Midusima (1939) of a large number of intervarietal hybrids of cultivated rice (*Oryza sativa*). Within this species these workers found that the F_1 hybrids between certain varieties had pollen and seed sterility quite comparable to that of interspecific hybrids. The F_2 progeny of semisterile F_1 hybrids showed a wide range of variation in sterility, as would be expected if multiple factor inheritance were present. The authors state that this segregation for fertility is independent of that for morphological differences, but do not give data to support this statement. However, the lack of correlation between visible intervarietal differences and their intersterility is evident from the fact that some of the most different varieties are nearly or quite infertile, while others, very similar in appearance, give highly sterile F_1 hybrids. This is shown partly by the authors' chart (Fig. 4, p. 231), and was brought out by a further analysis of their data made by Stebbins (1942a). This analysis revealed the additional fact that some of the most striking examples of morphologically

different races which are interfertile and of races which closely resemble each other in appearance but are intersterile are combinations including a race from some center of rice cultivation, such as Hawaii or South America, far from the original home of rice. These varieties are undoubtedly the result of extensive artificial hybridization and selection. This may be responsible for their behavior and suggests that the whole situation in rice has been greatly altered by its long history as a cultivated plant. It is possible that in the wild progenitors of rice, the visible differences and the partial intersterility barriers were correlated with each other, but that artificial selection has produced unnatural recombinations of the genetic factors responsible for these two types of differences.

The last two examples represent apparent exceptions to the lack of correlation between interspecific differences and hybrid sterility. Lamprecht (1941, 1944, 1945a,b) found that from the highly, but not completely, sterile F_1 hybrid between *Phaseolus vulgaris* and *P. coccineus* ("*P. multiflorus*"), lines could be isolated in later generations which, with two exceptions, represented recombinations of all the morphological characteristics separating the two species. These two exceptions (the position of the cotyledons of the germinating seedling, whether hypogeous or epigeous, and the character of the stigma surface) he considered to be characters determined by true species-separating genes. According to his interpretation, genes for the *coccineus* type of cotyledons and stigma cannot survive in *vulgaris* plasma, and, conversely, genes for the *vulgaris* condition of these two characteristics are inviable in the plasma of *P. coccineus*. To explain this situation, Lamprecht developed a new theory about the nature of gene synthesis. This theory needs verification on more carefully controlled material before it can be accepted, and, in view of the example to be presented below, it may not be necessary.

A parallel example is that of *Godetia amoena* and *G. whitneyi*.¹ Hiorth (1942) found that all strains of *G. amoena* found south of the Golden Gate, in central California, were interfertile and constituted the true genetic species, *G. amoena*. On the other

¹ Dr. Harlan Lewis (unpublished) has shown that these species names are incorrectly used by Hiorth and Häkansson. The correct name for the northern species called by them "*G. whitneyi*" is *G. amoena*, while the species which they call *G. amoena* is actually *G. rubricanda*.

hand, all strains classified by systematists as either *G. amoena* or *G. whitneyi* and originating from north of the Golden Gate and northward to British Columbia were likewise wholly or partly interfertile and constituted the species *G. whitneyi*. On the other hand, crosses between strains of *G. amoena* from south of the Golden Gate and *G. whitneyi* invariably form highly sterile hybrids, with a seed fertility of about 3 percent. Both species have the haploid chromosome number $n = 7$, and the F_1 hybrid has good chromosome pairing, usually forming six bivalents and two univalents.

Hiorth found, as did Lamprecht in *Phaseolus*, that, by means of backcrossing, most of the morphological characters peculiar to *Godetia whitneyi* could be transferred to the genetic background of *G. amoena*, and vice versa. A striking exception was the most distinctive difference between the two. Both species usually have a dark colored spot on their petals, although in both there occur genotypes with a recessive allele for the absence of spot. In *G. amoena*, however, the spot is always located at the base of the petal, while in *G. whitneyi* it is in the middle. Hiorth was at first unable to transfer the gene F^x , for central petal spot, from *G. whitneyi* to *G. amoena*, or the corresponding allele F^b , basal petal spot, from *G. amoena* to *G. whitneyi*. F^b plants were obtained in the backcross of the F_1 hybrid to *G. whitneyi*, but these were highly sterile. In one line, however, partly fertile F^b plants were recovered in the fourth backcross generation. These were analyzed cytologically by Häkansson (1947) and were found to have, instead of seven pairs, six pairs plus a chain of three chromosomes, one of them smaller than the other two. Among the progeny from one of these plants was found a fertile plant having seven pairs of chromosomes, which bore the F^b gene. The most plausible explanation of this situation, given by Häkansson, is as follows. The chain of three in the partly fertile F_4 plants consisted of one normal *whitneyi* chromosome, which is pairing at one end with a normal chromosome of *G. amoena*, and at the other with a fragmented chromosome of this species. The gene F^b is located in the normal *G. amoena* chromosome, which also contains factors causing sterility in combination with the chromosomes of *G. whitneyi*. The effect of these sterility factors is counteracted by the presence of the small chromosome from *G. amoena*,

which through segregation to the same pole of the two end chromosomes of the chain is often included in the same gamete with the F_b chromosome. The fertile F^b plants with seven pairs originated through crossing over between the chromosome bearing F_b and the normal one from *G. whitneyi*, thus transferring to the *G. whitneyi* genome the F^b factor without the sterility factors originally linked to it.

These experiments suggest that in both *Godetia* and *Phaseolus* the factors, causing the morphological differences between the species are different from those responsible for interspecific sterility. The apparently intimate association between certain morphological characteristics and the sterility barriers is one of genetic linkage, which can be broken by crossing over, provided enough offspring are raised.

SOME TYPICAL PATTERNS OF ISOLATING MECHANISMS

Although the complete analysis of the isolating barriers which separate species has been performed as yet in only a relatively small number of genera, nevertheless the data already available show that the principal isolating mechanisms acting to separate the species recognized by the systematist vary considerably from one group to another. This variation, furthermore, is not haphazard, and certain patterns of genetic species differences are becoming recognizable. These patterns appear to bear some relationship to the growth habit and distribution of the groups concerned.

We can recognize first a group of genera in which barriers of incompatibility, hybrid inviability, and F_1 hybrid sterility are relatively weakly developed. The most closely related species are separated largely by ecological or seasonal isolation and by inviability of F_2 segregates. Into this group fall *Pinus*, *Quercus*, *Larix*, *Cistus*, *Ceanothus*, *Wyethia*, *Aquilegia* (which also has some characteristics of the second group), some sections of *Aster* and *Solidago*, *Platanus*, *Populus*, *Rhododendron*, *Coprosma*, *Catalpa*, *Castanea*, and (except for the polyploid complexes) *Vaccinium*. It will be noted that these genera are mostly woody and are all long-lived perennials.

The second group consists of plants with flowers highly specialized for insect pollination and with mechanical isolation

strongly developed. Here are found the Asclepiadaceae and the Orchidaceae, mentioned earlier in this chapter. More cytological and genetic studies on these families are, however, needed. In some temperate genera of Orchidaceae, differences in chromosome numbers apparently provide the most important isolating mechanisms (Hagerup 1944).

A third group may be recognized in three genera of the Solanaceae, *Solanum* (diploid species), *Lycopersicon*, and *Datura*. In all of these, hybridization under natural conditions is often difficult or impossible, even when the species are closely similar in external morphology. On the other hand, the F_1 hybrids, when formed, tend to be sterile in spite of good chromosomal pairing and to yield sterile allopolyploids. This indicates the predominance of genic sterility, as well as incompatibility and hybrid inviability.

In the fourth group we may place those genera with an essentially "normal" relationship between amount of difference between species in external morphology, in crossability, in hybrid sterility, and in the chromosomes. Here belong *Layia*, *Allium*, *Lilium*, the diploid species of *Nicotiana*, *Gossypium*, and *Paeonia*, and probably many others. In them, several different types of isolating mechanisms are operating with about equal force.

A fifth group may be recognized in two genera: *Aegilops* (Kihara 1940, Aase 1935, Sears 1941a,b) and *Godetia* (Hiorth 1941, 1942, Häkansson 1941, 1943b, 1946a, 1947), in which an extraordinary amount of chromosomal differentiation has taken

place between nearly every species, even those which are morphologically rather similar. In them, nearly every interspecific hybrid has highly irregular chromosome behavior at meiosis, and allopolyploidy is common. In these genera, moreover, closely related species differ markedly in the external morphology of their chromosomes. This group is the direct antithesis of the one first mentioned.

The sixth, seventh, and eighth groups are those in which a large proportion of the barriers between species are due to differences in chromosome number. They may form extensive polyploid series, as in *Rosa*, *Rubus*, *Salix*, *Viola*, and most genera of grasses, or the numbers may be an aneuploid series, as in *Erophila*, *Carex*, *Scirpus*, *Iris*, and *Sipa*. Further barriers may be added by the

presence of apomixis, as in *Antennaria*, *Hieracium*, *Arnica*, *Potentilla*, *Poa*, and *Calumagrosis*. These genera will be discussed in later chapters dealing particularly with polyploidy and apomixis.

These groupings are only approximate, for purposes of orientation, and are certainly not mutually exclusive. Several of the genera mentioned above under one group have some characteristics of other groups. And still other genera exist within the limits of which are found species groups falling into most of the categories mentioned above. The most notable of these is *Crepis* (Babcock 1947). Species of this genus kept apart chiefly by geographical, ecological, or seasonal isolation are *C. pulchra-pulacea* and *C. foetida-thomsonii-eritreae*. Strong barriers of hybrid incompatibility or inviability between closely similar species are found in the case of *C. alpina-foetida*. The "normal" pattern of the fourth group mentioned above is perhaps the most common one in the genus, but *C. neglecta-fulginea* represent a species pair in which great differentiation in the chromosomes has taken place between morphologically very similar species. Polyploidy is present in several different species complexes, an aneuploid series of chromosome numbers is found in the genus as a whole, and apomixis is prevalent among most of the North American species. It is obvious that such genera as *Crepis*, in which many different speciation processes have been taking place in closely related evolutionary lines, are particularly good material for studies of the origin of species.

THE ORIGIN OF ISOLATING MECHANISMS

Although our knowledge of the nature of isolating mechanisms has been considerably increased in recent years, there is as yet no basis on which to erect more than a series of working hypotheses regarding the way in which they originate. The diversity of these mechanisms, as well as the fact that different ones are prominent in different groups of animals and plants, suggests that more than one hypothesis will be necessary to account for the origin of these barriers in the various groups of organisms.

The basic hypothesis held by many zoologists, which has been recognized since before Darwin's time and has recently been elaborated by Dobzhansky (1941, pp. 280-288), Muller (1942),

and Mayr (1942, pp. 154-185) is that in sexually reproducing, cross-breeding organisms geographic isolation must precede the formation of any other isolating barrier. The theoretical argument in favor of this hypothesis is as follows. If two populations are occurring in the same habitat and cross-fertilization between their members is possible, then the only type of barrier which will be effective in keeping them separate will be one which will permit the formation of vigorous and fertile offspring in matings within population A or population B, but will cause the weakness or sterility of offspring from matings between A and B. This result can be achieved only through systems of complementary factors, which produce vigorous and fertile individuals when homozygous, but cause weakness and sterility when in the heterozygous condition. If such a barrier were to arise by mutation and selection within a continuous, panmictic population, the individuals in which these mutations first occurred, as well as many of their immediate progeny, would be heterozygous for them, and according to the hypothesis would be weak or partly sterile. This would cause such a strong selection pressure against the isolating factors that their establishment would be impossible. On the other hand, such pairs or larger groups of complementary factors could readily arise in isolated populations, through mutations at different loci. Dobzhansky has suggested the simple situation in which the initial constitution of both populations is aabb, and one of them becomes transformed to AAbb by the occurrence and establishment of a mutation, while the other becomes similarly transformed to aaBB. The single heterozygotes, Abb and aaBb, are assumed to be viable and fertile, but the double heterozygote, AaBb, is inviable or sterile.

This argument possesses much less force in plants than it does in animals. As recognized by Dobzhansky, it does not apply at all to species which are capable of self-fertilization. In them, the temporary isolation which would be needed for the establishment of complementary factor systems can exist between the individuals of a spatially continuous population. A single heterozygous individual can produce, by selfing, homozygotes for new sterility or incompatibility factors, which can then become established, if favored by selection or random fixation. Furthermore, as pointed out in Chapter V, a considerable proportion of the higher plants

which are regularly cross-fertilized are very long-lived and produce a tremendous excess of seeds, so that partial sterility does not place them at a great selective disadvantage. This is particularly true of the numerous species with efficient means of vegetative reproduction. In most of the higher plants, therefore, sympatric speciation is a theoretical possibility.

On the other hand, the available factual evidence points toward the rarity of speciation without previous geographic isolation, that is, of sympatric speciation, in plants just as in animals. The zoological evidence has been well discussed by Mayr (1942, 1947). In those groups of higher plants known to the writer, the relationship known as "Jordan's law" (Jordan 1905) usually holds, as was stated also by Abrams (1905), on the basis of his wide experience with the California flora. That is, the nearest related species to any given species population is found, not in the same area or in a very different one, but either in an adjacent geographic region or in a far distant one with similar climatic and ecological conditions. Fernald (1934) has likewise emphasized the initial importance of geographic isolation.

In support of this opinion, the relationships will be analyzed of some of the species pairs mentioned earlier in this chapter which were stated to be separated by ecological or seasonal barriers rather than by geographical barriers, and which have shown their close relationship by their ability to hybridize with each other. The genus first mentioned is *Quercus*. The species of this genus in eastern North America appear like a group which has differentiated sympatrically during the evolution of the deciduous forests of this region, but this appearance may be simply the result of the long and complex history of these forests and the present lack of geographic isolating barriers. On the other hand, the oaks of the Pacific coast of the United States have a pattern of distribution which agrees perfectly with Jordan's Law. Examples are the coast and interior live oaks, *Q. agrifolia* and *Q. wislizenii*; the four species of the "golden oak" group, *Q. chrysolepis*, *Q. palmeri*, *Q. vaccinifolia*, and *Q. tomentella*; the three evergreen scrub white oaks, *Q. dumosa*, *Q. dumosa* var. *turbinella*, and *Q. durata*; and the two large-leaved, deciduous white oaks, *Q. lobata* and *Q. garryana*. The members of these species groups do overlap in their ranges to some extent, but their patterns are such as to sug-

gest strongly that the members of each group were geographically isolated from each other when they became differentiated.

The next example cited of ecological isolation was in the genus *Ceanothus*. Here the evidence suggests the past occurrence of "double invasions" into the central coast ranges (cf. Mayr 1942, p. 173). The three species mentioned were *C. thyrsiflorus*, *C. papillosum*, and *C. dentatus*, all of which are entirely or largely sympatric. The nearest relatives of *C. thyrsiflorus*, aside from some forms which are certainly to be regarded as subspecies of it, are the rare *C. cyanus* of southernmost California, the insular endemic *C. arboreus*, and a relatively common subshrub of the north coast ranges, *C. parryi*. This group of species seems to be essentially mesophytic in character and may well have originated as subshrubs in coniferous forests. *C. papillosum*, on the other hand, occurs in its most extreme form (var. *roseanus*) in the chaparral formations of Southern California, and all of its characteristics suggest that it originated under the hot, dry conditions which prevail in these areas. *C. dentatus* shows some resemblances to *C. papillosum* and its relatives, but its prostrate habit and the character of its foliage suggest a closer relationship to a species more typical of the inner central and north coast ranges, *C. foliosus*, which also occurs in relatively dry, chaparral areas. Such a habitat may have been the ancestral one for *C. dentatus*. The present juxtaposition of these three species is best explained as a result of the extensive migrations known to have taken place during the Pleistocene pluvial period, plus the large amount of uplift and faulting which changed completely the topography and probably also the habitats available in the central coast ranges during that epoch.

The ecological and seasonal isolation described in the genus *Pinus* probably has a similar explanation. In this genus, the shedding of pollen is closely associated with the beginning of spring growth in the vegetative shoots. In *P. radiata*, both of these processes begin in late winter, which in central and southern California is the most favorable time for the beginning of plant growth. This species, therefore, is perfectly adapted to the climatic conditions of this region, to which it is endemic, and it almost certainly originated there. On the other hand, *P. attenuata* shows a pronounced winter dormancy, so that both anthesis and

the beginning of vegetative growth are delayed until April. This is associated with the fact that the largest, most luxuriant stands of *P. attenuata* are in the interior sections of northern California, and that in some localities it ascends the mountains to an altitude of 6,000 feet (1,800 m). In these regions it is exposed to heavy winter frosts and snowfall, so that its dormancy is under such conditions essential to its survival. These are probably the climatic conditions under which *P. attenuata* became differentiated from the ancestral stock of the closed-cone pines. The other late-blooming species of this group, *P. muricata*, is strictly coastal and not at present exposed to heavy winter frosts. But its main area of distribution is far to the north of the range of *P. radiata*, and it very likely became differentiated on the coast of northern California. These three related species of pines, therefore, probably owe their initial differentiation to ecogeographic isolation and have become associated together only recently, probably during the Pleistocene epoch.

In his discussion of the importance of isolating mechanisms in the evolution of the species of *Crepis*, Babcock (1947a, pp. 147–151) does not state specifically whether or not geographic isolation has usually preceded the formation of other isolating mechanisms. But he points out that of the 182 species which are closely enough related so that they are comparable, 41 percent are completely isolated from their nearest relatives, and many of the remainder more or less overlap, but are largely allopatric. Those which are truly sympatric, such as *C. reuteriana*, *C. palestina*, *C. pterothecoides*, and *C. pulchra*, in the Syria-Palestine region, occur in different ecological niches and are adapted to different growing conditions. Their present association, therefore, may be, as in *Ceanothus*, due to migration from different regions in relatively recent times. Babcock assigns major importance as factors in speciation to geographic isolation and to ecological isolation "brought about through migration, either vertical or horizontal, or both, into new environments." The latter type of isolation corresponds to the modification of geographic isolation which Mayr (1942, pp. 194–198) has discussed in relation to various species of insects and birds. In *Crepis*, therefore, eco-geographic isolation has certainly played a major role in the origin of species, and it is likely that in the majority of cases, if not uni-

versally, it has preceded the formation of other isolating barriers.

Although as a general theory the initial importance of geographic or ecogeographic isolation is supported by most of the evidence at present available, the adoption of this theory does not enable us to draw any conclusions about the way in which other isolating barriers arise in geographically isolated populations. And this is the paramount problem in connection with the origin of species. This problem has been brought nearer to solution in recent years by a number of working hypotheses concerning which factual evidence has been or can be obtained. These hypotheses are not mutually exclusive, and in view of the diversity and complexity of genetic isolating mechanisms and of the apparent fact that different ones among them have played the major role in various groups of animals and plants, there is good reason to believe that several different mechanisms have operated to produce the isolating barriers which separate species.

The first hypothesis, which has been specifically or implicitly held by a number of geneticists and has been elaborated particularly by Muller (1940, 1942), is that if two populations are geographically or ecologically isolated from each other for a sufficiently long period of time, differential and divergent processes of gene mutation will inevitably cause the formation of other types of isolating barriers. As a general theory, this hypothesis can no longer be supported, at least in plants. Earlier in this chapter the writer cited the examples of *Platanus* and *Catalpa*, in which species living in different continents and markedly distinct in external morphology are able to form vigorous and fully fertile hybrids. Evidence reviewed in Chapter XIV indicates that such species populations have been separated from each other since at least the middle of the Tertiary period, that is, for thirty million years or more. Even recognizing the fact that a single generation in these species of trees may occupy scores or hundreds of years, one would be inclined to assume that if the origin of internal isolating barriers were inevitable in these isolated population systems, it would have occurred during this period of time. And the two instances cited could be matched by several others. The inevitable effects of genetic divergence produced by mutation and natural selection in geographically isolated population systems cannot account for more than a part of the isolating barriers now existing, and have

had relatively little to do with most types of hybrid inviability and sterility. Further evidence in favor of this conclusion is provided by the genetic independence of the factors responsible for hybrid sterility from those producing the visible differences between species, which was discussed earlier in this chapter.

Nevertheless, some isolating barriers are the direct result of evolutionary divergence in isolated populations. This is particularly true of ecological and seasonal isolation, as is evident from the examples cited above in *Quercus*, *Ceanothus*, and *Pinus*. Barriers caused by inviability of F_2 segregates from interspecific hybrids, and perhaps some examples of inviability of F_1 hybrids, may also be ascribed to this cause. As pointed out by Muller, the mutations responsible for differences in external morphology also cause differences in the rates of developmental processes. Therefore, new recombinations of these mutations may produce disharmonious systems of growth processes and, therefore, weakness or inviability of the hybrid product. The frequency with which this happens cannot be estimated at present, but the evidence presented above, as well as several other considerations (cf. Dobzhansky 1941, p. 284), enables us to say with some certainty that its occurrence is not inevitable.

Two hypotheses have been advanced which postulate natural selection as the guiding force in the establishment of isolating barriers. The first is that suggested by Fisher (1930) and further elaborated by Sturtevant (1938) and by Dobzhansky (1941). The basic assumption of this hypothesis is that, in the words of Dobzhansky (1941, p. 285), "gene recombination in the offspring of species hybrids may lead to formation of individuals with discordant gene patterns, the destruction of which entails a decrease of the reproductive potentials of the species whose members interbreed." The species concerned are viewed as systems of partially isolated and competing subpopulations, with two or more species occurring sympatrically. Interspecific hybridization, wherever it occurs, will produce many sterile or inviable products, and so will reduce the reproductive potential of the two subpopulations participating in it. If, therefore, genes arise in any subpopulation which prevent the occurrence of such interspecific hybrids, such genes will immediately increase the reproductive potential, and so will not only be quickly established by selection within that

subpopulation but will also cause that unit to expand and to spread the isolating factors by means of migration pressure. That this hypothesis cannot be generally applied to plants is evident from two facts. First, numerous examples are now known of crosses between widely different subspecies and partly intersubspecies which yield hybrids and hybrid derivatives which not only show no sign of inviability or weakness, but in addition may have a positive selective value under certain environmental conditions. These will be discussed in the following chapter. Here we shall refer only to the example of the F_2 segregates from a hybrid between two physiologically very different subspecies, *Potentilla glandulosa reflexa* and *P. glandulosa nevadensis*, many of which are very vigorous and appear to have a high positive selective value in the three standard environments of Stanford, Mather, and Timberline (Clusen, Keck, and Hiesey 1947, 1948b). Second, equally numerous examples are known, in which barriers of hybrid sterility exist between species which appear never to have been in contact with each other.

Nevertheless, the hypothesis may have a particular application to the origin of certain types of barriers, especially mechanical isolation. The intricate mechanisms for cross-pollination found in such groups as the orchids and the asclepiads are most probably produced by the precise interaction of many different genetic factors. Recombination of these factors in hybrids might be expected to yield many types of disharmonious systems of floral structures, incapable of functioning together. In such groups, therefore, maintenance of the reproductive potential might depend on the evolution of flowers adapted for cross-pollination in a particular way or by particular species of insects, or on the evolution of colors or other recognition marks which would cause the pollinating insect to pass instinctively from plant to plant of one species, avoiding its different relatives, as appears to be the case in *Antirrhinum* (Mather 1947).

Whether the hypothesis of the direct action of natural selection can be extended to account for isolating barriers in plants other than mechanical isolation is doubtful. In animals, it has been applied most frequently to examples of sexual isolation, or instinctively controlled aversion between males and females belonging to different species. For explaining such barriers, it seems

to be a most likely hypothesis and fortunately is capable of experimental verification.

Of greater potential importance in plant evolution is the hypothesis that hybrid sterility, particularly of the chromosomal type, may be produced by genetic correlation between the genic or chromosomal differences responsible for this sterility and certain combinations of characteristics which have a positive selective value. Muller (1940, 1942) has suggested the possibility of direct correlations of this nature, which are due to the multiple action of the same genes on development and have been caused by phylogenetic changes in gene function. The reasons why this suggestion cannot be widely applied to plants are the same as those against the hypothesis of the inevitable origin of internal isolating mechanisms between populations isolated for long periods of time. Muller's second suggestion, that structural changes in the chromosomes which could produce chromosomal sterility in hybrids might be correlated with selectively advantageous characteristics through the genetic phenomenon known as position effect, is likewise of limited application to plants. In spite of the large number of chromosomal changes which have been studied in detail, position effect has only rarely been found in the plant kingdom.

A third type of correlation, that due to genetic linkage and the suppression of crossing over in structural heterozygotes, may have widespread significance in explaining the origin of hybrid sterility of the chromosomal type. This hypothesis was first developed by Darlington (1936a,b, 1940) and was discussed further by Muller (1940, 1942). If, for instance, an inversion of a chromosomal segment arises in an individual, then the particular combination of ten or a hundred genes which happens to be located in the inverted segment will be transmitted as a unit to all the offspring of that individual which receive the inversion-bearing chromosome. In the inversion homozygote, crossing over will have no effect on this combination; in the heterozygote, crossing over will be suppressed in the inverted segment, due to inviability of the gametes bearing the crossover chromatids. Now, if the gene combination thus united happens to have a positive selective value or acquires such a value through new mutations, the chromosome bearing it will be spread through the population by natural

selection. If this selective advantage is held in only a part of the natural range of the species, then two different chromosomal types will be preserved indefinitely within this species. A similar but even more effective way of building up a diversity of chromosomal types within a species is through the existence of a selective advantage of an inversion heterozygote. For instance, if a particular inversion bears the alleles abcdef, and the combination \overline{abcdef} has a selective advantage over such combinations as \overline{ABCDEF} , as well as any other type of heterozygous or homozygous combination, then the inversion heterozygotes will be preserved as such, and both chromosomal arrangements will become permanently established in the population.

These situations are now no longer purely hypothetical. The prediction of Darlington (1940, p. 145), that the presence in *Drosophila pseudoobscura* of several different inversion types with characteristic geographic distributions would be explained on this basis, has now been strikingly confirmed by the experiments of Dobzhansky (Wright and Dobzhansky 1946, Dobzhansky 1947a,b). To obtain similar evidence in any plant group is now possible, because no method is available of studying plant chromosomes in such detail as is possible in the salivary chromosomes of *Drosophila*. But there seems little reason to doubt that such situations exist, particularly in view of the fact that inversion heterozygotes are extremely common in species of *Paris*, *Paeonia*, and other genera.

Furthermore, translocations of chromosomal segments could become established through their effectiveness in linking groups of genes located on different, nonhomologous chromosomes. This could come about in two ways. In organisms like *Oenothera*, *Tradescantia*, and the North American species of *Paeonia*, in which pairing and chiasma formation chiefly occur near the ends of the chromosomes and are rare or absent in the regions near the centromere, genes located in the latter regions of two pairs of nonhomologous chromosomes between which an interchange has occurred will automatically become linked, as shown in Chapter XI. But even in plants with random distribution of crossing over, the same result can be achieved via small interstitial translocations or through independent translocations affecting the same

two chromosome pairs, as discussed on page 224. In either case there will arise the two interstitially located segments (marked x and y in Fig. 24), which, because they are located on different chromosomes from the segments homologous to them, will usually never be able to cross over and yield viable gametes. Furthermore, the two segments which were transferred by the original interchange will thereafter always be inherited together, since gametes in which they are separated from each other will be inviable. This type of interchromosomal linkage can be achieved only at the expense of the occasional formation of hybrids with 50 percent sterility and a corresponding loss in the reproductive potential of the population, but as pointed out in the previous chapter and as will be further elaborated in the following one, this is not necessarily a serious selective disadvantage in many groups of plants.

The processes discussed so far explain only the establishment in a single species population of several selectively advantageous genotypes having different chromosomal rearrangements, and the partial cleavage of the population which this causes. The ultimate processes are necessarily those which cause the segregation of the chromosomal types into two or more groups, the members of each group being similar enough in their chromosomes so that they are essentially interfertile, and sufficiently different collectively from the members of other groups so that all intergroup matings result in partly or wholly sterile hybrids. Such a segregation is visualized most easily if the species population bearing different chromosomal types with differential selective values becomes broken up into several completely isolated subpopulations, each of which is subsequently exposed to a different kind of selection pressure.

Environmental changes that would promote this type of change in the population structure have occurred repeatedly in the history of the earth. As a typical example may be suggested the probable history of a forest-loving species living in the western United States during the period of mountain building which took place in the latter part of the Tertiary period. In the middle part of this period, during the Miocene epoch, forests of a mesophytic type, containing several different species of deciduous trees, as well as conifers, were widely distributed, and herbaceous plants adapted to this type of habitat could have formed more or

less continuous populations over large areas. But the uplift of the Sierra Nevada, the Cascades, and the lesser mountain ranges of the Great Basin cut off the interior of the continent from the moisture-bearing winds from the Pacific and caused the forests to become distributed along the moister, windward sides of the mountain ranges. Forest-loving species thus became restricted to a series of narrow bands, separated by many miles of inhospitable savanna, steppe, or desert country. Furthermore, the blocking off of these ocean winds from the interior undoubtedly caused a great change in temperature relationships, producing much greater winter cold and summer heat in the interior than near the coast. Any forest-loving species which could evolve in response to these changes would therefore not only become segregated into several isolated populations, but in addition the surviving individuals in each population would be forced to evolve in a different direction from that taken by those of other populations. If the ancestral, continuous population had possessed different chromosomal arrangements, some harboring gene combinations adapting the plant to relatively slight temperature changes and others favoring the adaptation to extreme conditions, then the process of isolation and selection would automatically break up the original species into several new species, which would be separated by the internal isolating mechanism of hybrid sterility as well as by geographic isolation.

The hypothesis just reviewed, therefore, postulates that the possession of different chromosomal arrangements associated in a particular way with adaptive gene combinations may preadapt the species to the formation of new species under the pressure of geographic isolation and differential selection. The most valuable evidence which could be secured in favor of this hypothesis would be the demonstration of the existence of chromosomal types with differential selective values in a species population which appeared to be in the process of breaking up. The information obtained by Hiorth (1942) and Håkansson (1942, 1944b, 1946a) about *Godetia whitneyi* suggests that this species may be such an example.

The next hypothesis, advanced by Wright (1940b), suggests that sterility-producing chromosomal or genic differences which are neutral in their selective value may become established by

random fixation in species populations while they are passing through "bottlenecks," or periods when selection has caused them to become much reduced in size. There seems little reason to deny that this could happen, provided the population became sufficiently reduced in size for a long enough period of time. Such a process could explain any internal isolating mechanisms which might be found between species of some oceanic islands living under a relatively uniform environment and of necessity isolated from each other. In continental species, however, there is some question as to whether isolation would often be long enough and the populations small enough so that an effective barrier could be raised. Nevertheless, random fixation could in many cases speed either the direct or the indirect action of natural selection in evolving isolating mechanisms, just as it could in respect to genetic factors affecting the visible differences between species, as discussed in Chapter IV.

The hypothesis that "bottlenecks" have played an important role in the origin of hybrid inviability and sterility is supported by the grouping of genera according to the types of isolating mechanisms found in them, as suggested in the previous section of this chapter. The genera placed in the first group, and characterized by the predominance of ecological and seasonal isolation, as well as inviability of F_2 segregates, are predominantly woody, long-lived, and usually exist in the form of large populations which are freely panmictic. Such trees as pines and oaks tend to dominate their habitats and are less sensitive to small changes in their environment than are most of the smaller herbs. Furthermore, isolation of such populations in time would have to be relatively much longer as compared to most herbs, in order to produce the same period of isolation in terms of generations. It is not surprising, therefore, that in groups of this type the isolating barriers are relatively poorly developed in relation to the morphological and ecological diversity of the individual species, and that they consist chiefly of those barriers which are produced most easily by the direct action of natural selection, without the aid of drastic changes in the size and structure of the populations or of the establishment of different chromosomal types.

On the other hand, the two genera placed in the group at the other extreme, namely, *Aegilops* and *Godetia*, are herbaceous and

consist entirely or mostly of annuals. Nearly all of their species occupy pioneer habitats in disturbed ground, and many of them are very local in their distribution. Others, which are now widespread as weeds, were probably also much restricted before the advent of man. Such groups have undoubtedly gone through many changes in population size and have been subjected to many alterations in their environment, to which they may have been particularly sensitive. They are therefore groups in which the processes hypothesized above as most important in the origin of internal isolating barriers would be expected to proceed most rapidly. In addition, certain species groups in other genera which have been used in this chapter as examples of the strong development of barriers of hybrid inviability and sterility, such as *Crepis neglecta fuliginosa* and certain species of *Hemizonia* and *Layia*, are likewise annuals living in disturbed, unstable habitats. On this basis, we should expect that the presence in a genus of many polyploid species containing large numbers of morphologically and ecologically differentiated subspecies would be an indication that in this genus the most effective speciation processes were proceeding at a relatively slow rate. Rapid speciation would be indicated by the presence of many relatively constant, closely similar species with restricted and adjacent distributions, but separated by well-developed barriers of hybrid inviability or sterility.

The final hypothesis concerning the origin of internal isolating mechanisms was suggested by the present writer (Stebbins 1942a, 1945). This is that new isolating mechanisms may be compounded from old ones through the segregation of fertile derivatives from partly sterile interspecific hybrids. This hypothesis will be discussed in the following chapter, as one of the most important effects of hybridization. Experimental evidence now available and still being obtained suggests that isolating mechanisms can arise in this manner, and that in some plant genera this method may have played a rather important role. It can be particularly effective in originating new barriers of chromosomal sterility in species groups with predominant self-fertilization.

Our present knowledge of isolating mechanisms, therefore, supports the statement given at the beginning of this chapter, that descent with modification and the origin of species are essentially different processes. Furthermore, since six or more different

hypotheses which have been suggested to account for the origin of interspecific isolating mechanisms can all be supported by some evidence in the case of certain particular groups of animals or plants, we can conclude that the evolutionist must deal, not with a single process, the origin of species, but with several different processes, the origins of species.

CHAPTER VII

Hybridization and Its Effects

THE ROLE of hybridization in evolution has been one of the most controversial topics in the whole field of evolutionary study. Some authors, particularly Lotsy (1916, 1932) and Jeffrey (1915) have assigned a dominant role to this process. Other botanists, particularly systematists occupied chiefly with identification, classification, and the compiling of generic monographs and local floras, have been reluctant to recognize the existence of more than a very small number of hybrids in nature, and have assigned relatively little importance to these. Many zoologists, also, such as Mayr (1942), failing to detect evidence of hybridization in their material, have minimized its evolutionary effects.

The evidence to be presented in this chapter indicates that the true situation, at least as far as the higher plants are concerned, lies somewhere between these extremes. Even if we use the term hybridization in its broadest sense, namely, the crossing of any two genetically unlike individuals, the greatest possible importance we could assign to it would be as a third major evolutionary process, recombination, with an importance not exceeding that of mutation and selection. If we restrict the term to its most commonly accepted usage, namely, crossing between individuals belonging to reproductively isolated species, its importance must be considerably less. On the other hand, careful studies of numerous groups of higher plants from the cytogenetic as well as the systematic point of view have shown definitely that in many of them interspecific hybrids are rather common in nature. Furthermore, these hybrids have frequently given rise to offspring of later generations which have considerably modified the pattern of variation in the groups to which they belong. In fact, the accumulating evidence may make possible the generaliza-