Speciation in Flowering Plants

Rapid chromosome reorganization in marginal populations is a frequent mode of speciation in plants.

Harlan Lewis

Everyone knows there are species, although no one has yet proposed a definition acceptable to all biologists, nor is anyone likely to. Nevertheless, there seems to be no disagreement as to what the process of speciation involves. Since species differences, no matter how species are defined, can be maintained only by barriers of some sort to gene exchange, speciation obviously involves the establishment of such barriers. Speciation can be discussed, therefore, without a precise definition of the end product. Most biologists, however, are likely to ask what there is to discuss, for not only has the process been described repeatedly in recent years, but nearly all treatments of the subject have been in essential agreement since Goldschmidt's arguments for speciation by "hopeful monsters" (*1*) were refuted a quarter of a century ago.

Evolution leading to speciation, except speciation by polyploidy, is generally considered to be a gradual process that includes a succession of stages, from the formation of barely detectable ecological or geographical races to ecogeographic differentiation readily recognizable at the taxonomic level of subspecies. Subsequent development of reproductive barriers carries differentiation to the level of species. Speciation in this manner undoubtedly occurs in flowering plants, for in some genera all stages of the sequence can be found. On the other hand, the normal mode of speciation in many genera appears to have no direct relation to the formation of ecogeographic races or subspecies. It was also Goldschmidt's thesis (*1*) that speciation is a process distinct from subspeciation. He postulated that speciation results from the establishment of systemic mutations that involve chromosome reorganization and change the reaction norm of the entire genotype, but he was unable to substantiate this mechanism convincingly. I agree with him, however, that evolution involving chromosome reorganization is very different from that resulting from gradual accumulation of genic differences. This is not to say that chromosome rearrangements never become established in the course of ecogeographic differentiation, but rather that chromosome reorganization frequently is not a gradual one-by-one process. In the case of rapid chromosome reorganization, speciation is not an extension of ecogeographic race formation.

Although I wish primarily to discuss very rapid, saltational speciation, which in recent years has generally received little attention, it is useful, for comparison, to consider first some of the general characteristics of species and speciation in groups of flowering plants when little or no chromosome reorganization is involved. My purpose is to emphasize that there are two distinct modes of speciation, that the products are not equivalent in their relationship to one another, and that they set the stage for different patterns of subsequent evolution. I realize that some of my statements are oversimplifications and that other examples would show that the alternatives are not always as sharply defined as they are presented here.

Gradual Speciation

Evolutionary divergence in groups of flowering plants in which chromosome arrangement remains essentially constant follows the generally accepted rules of geographical speciation, except that barriers between the resulting species are usually incomplete and remain incomplete for indefinite periods of time, even when the species are sympatric (that is, grow within normal pollinating range of one another). Complete barriers, when they occur, are more often accidental by-products of evolution that the result of selection for reproductive isolation. Closely related species that may have gradually diverged often occur in different geographical areas; if they are sympatric they usually occupy distinct habitats. Except for selection by the habitat and some degree of spatial separation that goes with ecological differentiation, barriers to gene exchange between species that are in contact are mostly of the sort that decrease the rate of hybridization but do not restrict gene recombination within hybrids that are produced; they depend on pollinator preference, structural differences in the flower that impede pollen transfer, or a seasonal difference in flowering time, all of which require constant selection to remain effective unless, of course, they completely eliminate hybridization.

Gene exchange is seldom eliminated by natural selection because barriers between species, other than ecological differentiation, are evolutionarily superfluous and incidental to the continued adaptedness of the population concerned. Two examples will serve to illustrate these points: One concerns the barriers between two species of columbine that hybridize extensively where they come into contact; the other, two species of sage that are in contact over a wide area with very little evident hybridization.

Aquilegia formosa and *A. pubescens* are conspicuously different; the former has pendulous bright-red flowers with relatively short spurs, whereas the flowers of the latter are larger, erect, cream or pale in color, and have relatively long spurs. Both occur in the Sierra Nevada of California where *A. formosa* characteristically grows along shady streams and *A. pubescens* on open rocky slopes at generally higher elevations. The careful observations of Grant (*2*) have shown that the red-flowered species is normally pollinated by hummingbirds and the other species by hawk moths. Although the morphological and ecological modes are extremely different, the distribution of columbines is continuous in many places and, where talus spills onto a meadow or a stream flows down a rocky slope, in the area of overlap a wonderful array of morphological recombinants invariably is found. Hybridization is undoubtedly

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The author is professor of botany and dean of the Division of Life Sciences, University of California, Los Angeles.

frequent, and the hybrids produce progenies which in turn produce an abundance of seeds. The only barriers to gene exchange appear to be selection by the habitat, propinquity of similarly adapted individuals, and pollinator preference.

Although no one has ever questioned their specific status, the relationship between these columbines is directly comparable to that between the ecological races that have been designated as subspecies in the well-known studies on *Potentilla glandulosa* (*3*) (for example, *P. glandulosa hanseni*, the meadow race, and *P. glandulosa reflexa*, on the adjacent drier slopes) except that the floral differences in *Potentilla*, which are just as numerous but less conspicuous, have not been shown to be associated with pollinator preference. But even subtle differences in floral morphology are not essential to the maintenance of adaptation to different adjacent habitats, as is indicated by Turesson's classical studies (*4*) of ecotypic differentiation. Turesson has shown in several species, for example, that populations growing on the sea cliffs, and differing conspicuously in leaf shape, habit, and other vegetative traits from populations on adjacent dunes, maintain their distinctive characteristics although the differences are genetically determined and there are no barriers to interpopulational hybridization, other than a negligible spatial separation.

The evidence leads to the conclusion, therefore, that the conspicuous morphological difference between the columbines, which is maintained by pollinator preference, is essential only to the maintenance of species-distinguishing characters and is incidental to ecological adaptation, neither enhancing nor preserving it. One might ask then: How did the difference arise in the first place? How is it maintained despite extensive hybridization? And why has hybridization not been further reduced by the evolution of additional barriers?

The first question, of course, can never be answered precisely, but it is not difficult to visualize that populations of columbines in different areas, whether widely separated or adjacent, would become adapted to different pollinators if the pollinator which they initially had in common became rare in one of the areas. Since continued adaptation of any population depends on production of adapted progeny, selection for floral traits that will attract the same pollinator to similarly adapted individuals follows automatically. Consequently, differences in floral morphology between ecologically differentiated populations, once established, will be maintained indefinitely by selection, regardless of rate of hybridization.

Additional barriers to hybridization have not evolved by natural selection because, in some areas, hybrids are apparently as well or better adapted physiologically than either species. Furthermore these areas are at the points of contact where hybridization is most prevalent. As long as hybridization results in the production of adapted progeny, hybridization will not be eliminated by selection. If the area to which hybrid derivatives are adapted is small, the populations within that area may remain highly variable in morphology because newly formed hybrid combinations continually become established. On the other hand, should the intermediate habitat expand in area, or if an extensive intermediate habitat becomes available, a third species may arise which is morphologically as uniform as either of the parents (for example, *Delphinium gypsophilum*) (*5*) and which may be adapted (for example *Penstemon spectabilis*) to a third and very distinct pollinator (*6*).

Barriers between sympatric species are generally more effective in reducing hybridization than one finds in the case of the columbines, and maintenance of specific status may appear, erroneously, to be less dependent on ecological adaptation. This is the case in *Salvia*. *Salvia mellifera* and *S. apiana* are morphologically so distinct in both vegetative and floral traits that they are placed in different subsections of the genus (*7*). Both species are conspicuous components of the soft shrubby coastal sage association, which covers much of southern California at lower elevations. Throughout this large area the two species are frequently in close contact or grow together but show very little evidence of hybridization in undisturbed habitats. The barriers to hybridization are several (*8*): The species are preferentially visited by different bees; the small-flowered *S. mellifera* by various small solitary bees in addition to the introduced honey bee; and the larger-flowered *S. apiana* by large carpenter bees or bumblebees. When individuals of both species are visited by the same bee, pollination usually does not occur because of the great difference in flower structure. The two species also have modally different flowering seasons. Together these barriers undoubtedly reduce hybridization to a low frequency. In addition, hybrids that do become established set relatively few seeds. Despite the interaction of all these barriers, however, hybrid swarms consisting of a spectacular array of morphological recombinants are found in habitats that have been extensively disturbed by man (*9*). This can only mean that the essential barrier between these extremely divergent species is that hybrids and hybrid derivatives are not adapted to the undisturbed habitat (*10*); the other barriers are incidental.

The *Salvia* example differs from *Aquilegia* in only one important respect, namely, there is at present no natural habitat consistently available to the hybrids. Since production of hybrid seeds that cannot become established would reduce the reproductive potential of the plant that produced them, barriers that greatly reduce the rate of hybridization would be expected to develop. The question remains, however, why complete barriers to hybridization have not evolved. This has not happened, I believe, because the barriers that have developed reduce hybridization to a point where the loss in reproductive potential from the formation of hybrids is no greater than loss due to vagaries of the environment on which selection cannot act.

Discussions of speciation generally focus on the stage of divergence at which populations become genetically independent or "reproductively isolated," largely, I suspect, because this stage offers an objective basis for delimiting species. With this emphasis, it has become customary to equate all barriers to gene exchange, whether they merely reduce the frequency of hybridization and hence the rate of recombination or whether they prevent certain combinations from occurring, under the rubric "isolating mechanisms." With respect to flowering plants, this orientation has led many to the rationalization that morphologically well-defined species such as those in *Aquilegia* and *Salvia* must be reproductively isolated, although obviously they are not. On the contrary, the success of these and many other genera, measured in terms of morphological and ecological diversity, is due in large measure to the absence of reproductive isolation; the evolutionary potential of such groups is not limited by the genetic resources of each species alone.

Speciation by Saltation

Evidence that speciation occurs as a result of saltational reorganization of the chromosomes, which I shall call saltation, comes from the observation that adjacent populations, which are very similar in morphology and in ecological adaptation, may differ greatly in chromosome arrangement and sometimes in basic chromosome number. Because of multiple chromosomal differences, hybrids between such populations have very low fertility and would ordinarily be called sterile. Populations intermediate in chromosome arrangement do not occur. The first indication of saltation I had, for example, came from unexpected results following experimental hybridization between two diploid populations of *Clarkia* that were so similar that anyone would have considered them conspecific (*11*). Since they came from ecologically comparable sites less than a mile apart, I was surprised to find that the hybrids were essentially sterile because of multiple differences in their chromosomes. At the same time, one of these populations produced fertile hybrids with other populations which differed in a number of vegetative and floral traits, including some populations that had been placed in another species.

A similar lack of correspondence between morphological divergence and hybrid fertility has since been found in other sections of *Clarkia* (*12, 13*) and is also known to occur in genera belonging to other families (for example, *Haplopappus*, and *Holocarpha* in the Compositae) (*14*). The most obvious characteristic these diverse genera have in common is that they are mostly annuals. One reason, although not the only one, is that the initial products of saltation are morphologically and ecologically so similar to parental populations that hybridization is necessary to detect the barriers between them, and annuals are notably useful for studies involving systematic hybridization between populations. I am convinced, however, that saltation also occurs in woody plants, although the evidence, as discussed in a later section, is more indirect.

Morphologically indistinguishable or very similar sympatric species are generally called sibling species, with the obvious implication that they are derived from a common parent (*15*). Following saltation, however, the relation between species is clearly that of parent and offspring, although two or more species independently derived from one parental species may appropriately be thought of as sibs. In *Clarkia*, for example, Mosquin (*16*) has evidence that two species have been independently derived from *C. mildrediae*, and Vasek (*17*) has found four diploid species derived from *C. unguiculata*; in *Chaenactis* (Compositae), Kyhos (*18*) has demonstrated that two desert species, *C. stevioides* and *C. fremontii*, have been derived from a more coastal species, *C. glabriuscula*. The detailed analysis of chromosomal relationships in *Chaenactis* also illustrates the precision with which the direction of evolution can be demonstrated cytologically.

Another characteristic of saltation is that the derivative and parental species are unable to grow together in mixed populations, even though they are ecologically very similar, unless a barrier subsequently evolves that prevents or greatly limits hybridization. *Clarkia biloba* and its derivative *C. lingulata* illustrate this clearly. These species, which differ in external morphology only in the shape of the petal, hybridize freely when they are in contact, but the hybrids have very low fertility. In experimentally mixed populations, one or the other species was rapidly eliminated, depending on their relative abundance (*19*).

The species in lower frequency produced a larger proportion of hybrids, which in turn decreased its own relative frequency. As its numbers decreased, elimination was accelerated by the ever-increasing proportion of hybrids in its progenies. The net effect of such elimination is mutual exclusion. Once either species becomes established in a particular site, any migrant from the other is eliminated by hybridization regardless of which species is better adapted to that site. At the one place where *C. biloba* and *C. lingulata* come into contact in nature, the overlap is a narrow band about 3 meters broad which represents the limit to which one can penetrate the territory of the other before it is eliminated by hybridization. Mutual exclusion also accounts for the patterns of distribution between other morphologically very similar species of *Clarkia* and undoubtedly those in many other genera. In *Lasthenia* (Compositae), for example, two morphologically very similar species, *L. fremontii* and *L. conjugens*, occur in what appear to be comparable ecological sites close to one another but are not found growing together. Significantly, these species hybridize readily in cultivation, and the hybrids have low fertility due to chromosomal differences (*20*).

When the fertility of hybrids is very low, one would expect a strong positive selection for any factor that prevents hybridization in areas where hybridization is frequent. This has not occurred in the case of *Clarkia biloba* and *C. lingulata* described above, but the contact between these species is apparently very recent. In most instances, however, secondary barriers have developed in response to selection. For example, two very closely related species, *Clarkia williamsonii* and *C. speciosa*, which until recently were considered conspecific, readily form hybrids if the plants that are crossed come from populations far removed from the area where the two species grow together; if they come from within or close to the area where the species are sympatric, they cannot be crossed (*21*).

Mechanism of Saltation

The problem is to account for multiple structural differences in adjacent populations when plants heterozygous for one of these rearrangements have low fertility. For any one rearrangement to replace the original throughout a population, the new arrangement must increase in frequency, despite its effect on fertility, and produce homozygotes that are better adapted than the heterozygotes and the original homozygotes, unless replacement occurs by chance in a very small population. Reardless of how replacement occurs, its frequency must be very low, otherwise populations would frequently be found to differ in chromosome arrangement. If the probability of the replacement of one arrangement by another is low, the probability that it will recur independently in any given population four, six, or more times is effectively nil. Yet *Clarkia franciscana*, which consists of a single population that we initially included in *C. rubicunda* (*22*), differs chromosomally from that species by at least three translocations and four inversions, whereas *C. rubicunda*, which is morphologically and ecologically diverse, is chromosomally uniform throughout its entire geographic range (*13*). Were this the only example, we could dismiss it, for even the most improbable events can happen once.

There are, however, similar examples not only in *Clarkia* but apparently in other genera such as *Holocarpha, Lasthenia*, and *Allophyllum* (*14, 20*). The conclusion seems warranted, therefore, that multiple chromosomal differences, under some circumstances, arise and become established simultaneously. Simultaneous establishment is also a highly improbable event, but much less so than gradual accumulation within a given population because it need occur only once in any one of a vast number of populations during an indefinitely large number of generations.

Saltation is initiated by one or more individuals heterozygous for structural rearrangements that greatly reduce fertility. Such individuals, were they in any normal population, would have very little chance of contributing to the next generation, let alone of affecting the chromosome organization of the entire population. If, however, an individual which is nearly sterile is removed from competition with normal members of the parental population, its degree of fertility is of no consequence if it is able to produce any progeny at all. The probability of a progeny may be very small but, if the population does not become extinct, heterozygosity that reduces fertility will rapidly be eliminated. The resulting population may or may not have the original chromosome arrangement. If not, and if hybrids with the parental species have low fertility, the derivative population will be able to maintain itself against encroachment by the parental species, even though it comprises less vigorous, less fecund individuals.

A population consisting of only one individual is not essential to saltation and presumably could not have been the case in *Chaenactis* described above, because the parental species and both derivatives are self-incompatible (*18*). I wish to emphasize, however, that spatial isolation of one or very few individuals from the parental population is required and must be maintained until the derivative population is able to eliminate migrants through hybridization or to grow with them without hybridizing. The distance required for isolation may be very short, however, because the area over which seeds and pollen are normally dispersed is often very limited.

Spatial isolation of one or a few individuals can occur either by dispersal into a site not occupied by the species or by survival after all other members of the population have been eliminated. Both undoubtedly occur, but the latter, when due to catastrophic selection that suddenly eliminates nearly all of a population at the ecological limits of the species, is most likely to lead to saltational speciation. Individuals that survive an exceptional environmental extreme, such as unusual drought, find themselves in an open habitat that is ready to be repopulated and to which their progenies may be adapted with respect not only to the normal environment but also to an environmental extreme which may recur. Evidence that catastrophic selection rather than dispersal is associated with saltation in *Clarkia*, which occurs in semi-arid regions with highly seasonal rainfall, comes from the observations that all derivative species are adapted to a shorter growing season than their respective parents and that unusually short growing seasons, limited by water, have catastrophic effects on populations (*23*).

Regardless of how spatial isolation occurs, a more difficult problem is to account for multiple breakage of the chromosomes in the particular individuals that are isolated. It would be ridiculous to suggest that individuals that become isolated from the parental population are by chance heterozygous for several chromosomal differences. Excluding chance, however, leaves only two alternatives: either the factor responsible for isolation induces breakage, or isolation itself is conducive to breakage. Although the possibility cannot be excluded that an extreme condition producing catastrophic selection may also induce breakage, there are no data to indicate that it does. On the other hand, there is evidence for the second alternative.

Isolated individuals, or small groups of individuals, must inbreed if the population is to continue. As is well known, forced inbreeding of normally outcrossed individuals often results in progenies showing decreased vigor, abnormal development, or reduction in fertility. Of particular relevance to speciation by saltation is that inbreeding may lead to extensive chromosome breakage (*24*).

The significance of a sudden shift to intense inbreeding on the genetics of a population has been discussed in detail by Mayr (*15*) with respect to the role of small "founder" populations in speciation, and his arguments for "genetic revolution" are equally applicable to small "survivor" populations at the ecological limits of a species. Regardless of how a very small population may arise, adaptive gene complexes that emerge from the bottleneck of inbreeding must be preserved from disruptive recombination if they are to form the basis of a successful new species. Chromosome reorganization, of course, has this effect.

The frequency with which successful chromosome reorganization occurs after forced inbreeding undoubtedly varies tremendously from one group of organisms to another, but it need not be frequent to be highly significant. In *Clarkia*, for example, where at least 14 out of 28 diploid species have almost certainly had a saltational origin, only one successful reorganization throughout the countless marginal populations of the entire genus would be required every thousand generations or so, even if we assume that all 14 are more recent than the last glacial maximum.

In conclusion, the chromosomal barrier to gene exchange that results from saltation may be considered an accidental by-product of forced inbreeding, which has the effect of immediately establishing an irreversible genetic independence between parental and derivative populations with regard to a large portion of their respective genotypes. If hybridization between the resulting populations is frequent, secondary barriers that greatly reduce or eliminate hybridization would be expected to develop by natural selection. Immediate irreversible independence, which permits a genetically depauperate population to explore new sorts of genetic combinations in the course of reconstructing an adaptive genetic system, may lead to relatively rapid divergence from the parental populations. In contrast, slowly differentiating groups that retain the potentiality of interspecific gene exchange have far greater capacities to adapt in response to changes in the environment but, since they evolve more or less as a unit, are less likely to give rise to developmentally new adaptive modes.

Saltation in Woody Plants

Saltational reorganization of chromosomes is demanded as an explanation only when morphologically similar, adjacent populations differ chromosomally in such a way that piecemeal accumulation of the rearrangements is not a reasonable explanation. This is frequently the case among annual plants, as I have indicated, but appears to be rare and has not been demonstrated in woody plants. Nevertheless, there is evidence that leads me to believe that saltational speciation has played a significant role in the evolution of woody as well as herbaceous groups, particularly with respect to the initiation of divergence leading to categories higher than species.

This conclusion is based on comparative data, primarily basic chromosome numbers. The significance of basic numbers is twofold: (i) they are very conservative, particularly among woody plants where one frequently finds the same chromosome number throughout a family, subfamily, or other large taxonomic group. Even among herbaceous groups, basic chromosome number is generally constant throughout a genus or major sections of it, although the number is frequently different from one genus to another in the same family. In general, a difference in basic number is not characteristic of closely related species, although examples are known in which populations that have been considered taxonomically conspecific differ in basic number (*14*). It seems clear that change in basic chromosome number is not a normal consequence of gradual differentiation, but a rare event resulting from unusual circumstances. (ii) In nearly all instances in which a difference in basic chromosome number in plants has been studied in detail, gross differences in chromosome arrangement have been found in addition to those required to effect the change. This suggests that saltation has been involved.

The differences in chromosome number within some groups of woody plants in Australia, compared to those within groups of related woody plants in other parts of the world, are particularly instructive. Smith-White (*25*) has shown that the chromosome numbers of taxa endemic or nearly endemic in Australia, such as the Chamaelaucoideae (Myrtaceae) and Boronieae (Rutaceae), are much more diverse than those within comparable taxa in the same family outside Australia. The differences in number are characteristic of genera, not species, and the distribution of species in the same genus indicates that chromosome numbers have long been stable. He concludes, therefore, that the earliest flowering plants to migrate into Australia met with conditions conducive to chromosome change and that these conditions have not persisted. He also points out that availability of diverse new habitats unoccupied by angiosperm competitors provided an evolutionary opportunity for the more flexible lineages to expand into diverse habitats and has suggested that this was conducive to genetic experimentation, including change in chromosome number. Although I doubt that opportunity to expand is alone a sufficient condition for change in chromosome number, if one adds isolation of one or a few individuals by chance dispersal or catastrophic selection, it does become understandable. The latter means of isolation seems very likely because surely not all the tropical species of flowering plants that crossed the first bridgehead to Australia migrated steadily across the face of the continent without periodic setbacks from over extension of their range. After saturation of available habitats by angiosperms, however, a sudden reduction of a population of woody plants to a few individuals would not, ordinarily, have left a habitat open for a genetically depauperate derivative population to increase in numbers.

Conditions in Australia that resulted in chromosome changes, which now distinguish genera, were doubtless comparable to those that accompanied the much earlier expansion of flowering plants throughout the rest of the world. The changes that occurred at that time, however, may be reflected in the chromosomal differences we now perceive between families or other major taxonomic groups.

Summary

At the risk of gross oversimplification, I have contrasted two modes of speciation and compared the products, with the intent of emphasizing that speciation is not always an extension of gradual ecogeographic differentiation. The genetic relationship between species resulting from adaptation to persistent environmental differences is not comparable to that between species resulting from saltational reorganization of the chromosomes following a unique event that isolates one or a few individuals in an open habitat free from conspecific competition. Species resulting from gradual differentiation are characterized by incomplete barriers to gene exchange, which are maintained by selection and remain incomplete for indefinite periods of time even though the species are in contact. The potentiality of acquiring genetic variability from one another provides such groups of species with a high degree of adaptability.

Chromosomal reorganization, which is most likely to occur following a sudden change to intensive inbreeding in extremely small marginal populations, is an improbable event, and the resulting genetically impoverished population has an inauspicious future. Once established, however, such a population is able to exclude parental migrants while its genetic system is being reconstructed. The immediate genetic independence of the population should, however, permit novel gene combinations to become established which might provide the derivative population with an evolutionary potential very different from that of the parental species; the stage may occasionally be set for major divergence.

Saltational speciation in flowering plants is required as an explanation only for the relationships between particular populations of annuals that have been studied intensively. By reasonable extrapolation, however, it appears to be the prevalent mode of speciation in many herbaceous genera and to have had a significant role in the evolution of woody plants.

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Blood-Group Substances

In the ABO system the genes control the arrangement of sugar residues that determines blood-group specificity.

Winifred M. Watkins

The first divisions of blood into groups were based on differences among the antigenic substances on the surfaces of the red cells of a given species of animal. These antigens are distinguished by means of antibodies in serum that combine with the red cells. Blood-group characters are inherited according to simple Mendelian laws, and the antigens, believed to be products of allelic, or closely linked, genes, are classified together in blood-group systems (*1*). Inherited variations in serum proteins (*2*) and enzyme activities in both serum and red cells (*3*) are other factors now known to differentiate bloods within a species, but the blood-group substances discussed in this article are those related to the groups defined by the red cell antigens.

The first human blood-group system, the ABO system, was discovered by Landsteiner (*4*) as a result of his attempts to determine whether specific serological differences existed between individuals of the same species, and the importance of a knowledge of the ABO groups for the safe practice of blood transfusion was promptly recognized. The second blood-group system of outstanding clinical significance, the *rhesus* (Rh) system, was not discovered until 40 years later (*5*). In the intervening years two other systems, the MN and the P systems, were found from examination of serums from rabbits injected with different samples of human red cells (*6*). The antigens in the MN and P systems are of little clinical importance because the corresponding antibodies occur infrequently in man and seldom produce untoward transfusion reactions. At the time of their discovery, however, the inheritance of very few normal human characters was established, and the M and N blood-group factors were used as research tools in genetics and anthropology. With the outbreak of World War II there was a rapid expansion of blood transfusion services in many countries, and the consequent development of improved blood-grouping techniques, together with the vast increase in the number of blood samples examined, led to the discovery of many new blood-group systems and subdivisions of the existing groups. Since the end of the war studies on the serology and genetics of blood groups have continued to flourish; now some 14 human systems, which include over 60 different blood-group factors, are known (*1*).

Although from the point of view of the clinician it is not necessary to be acquainted with more than the serological relationships of the blood-group antigens and antibodies to carry out successful blood transfusions, it is of great importance to study their chemistry and genetics in order to understand the basis of blood-group specificity. The blood groups are defined by their serological properties; the antigens are identified by means of antibodies which may be derived from the same species as that in which the antigen is demonstrated, or from a different species. In the simplest instances specific combination of antigen and antibody causes red cells to clump or agglutinate; of equal importance in blood-group serology are the so-called "incomplete" or "blocking" antibodies that can be demonstrated only by more refined serological techniques (*1*). Chemical studies of blood-group antigens have not kept pace with the serological and genetical advances, but for the ABO and closely related Lewis systems a pattern of relationships between gene function, chemical structure, and serological specificity is beginning to emerge, and consideration of these systems is the principal topic of this article.

Water-Soluble Blood-Group Substances

Somewhat paradoxically the largest amount of information on the chemical basis of blood-group specificity has come from chemical and immunological examination of substances that are not derived from red cells. In the first three decades after the discovery of the ABO system, attempts to obtain blood-group active materials from red cells met with limited success. A and B substances could not be extracted from the cells with water or salt solutions, but active preparations were obtained by extraction with ethanol; the substances were therefore designated as "alcohol-soluble." The amounts isolated were very small, and reports on their chemistry were conflicting (*7*). Further impetus to chemical studies came from the discovery that, in humans, substances with A- and B-activity are present not only on the red cell, but they also occur in a water-soluble form in the tissue fluids and

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The author is Reader in Biochemistry (Immunochemistry), University of London, Lister Institute of Preventive Medicine, London, S.W.1.