

GENETICS AND THE ORIGIN OF SPECIES

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EDITOR'S PREFACE

IT IS fitting that the Columbia Biological Series should resume publication with a discussion of the same problem to which the first volume in this series was devoted. Forty years ago evolution was a problem in history, and in *From the Greeks to Darwin* H. F. Osborn traced the origins and development of the idea of gradual change as the means by which the world and its inhabitants had assumed their present forms. It was not only the ideas and theories which were studied as history; the facts of evolution itself were shown to be determined by changes in the past history of the earth. The essence of Darwin's theory was just this, and the efforts of biologists were concentrated on describing as completely as possible this record of the past.

But description and reconstruction fail to satisfy for long even the most ardent historians of nature and it was Darwin's second great service to have focused the attention of biologists upon the forces that caused the changes and particularly upon the problem of what agencies brought about and maintained the diversification of animals and plants into distinct species. His own theory of natural selection, by appealing to two occurrences of which the details were quite unknown—the origin of new variations in animals and plants and the perpetuation of these by heredity—served as a great stimulus and provided much of the motivation for biological research in the period which followed 1859. There were at that time, however, no reliable methods by which these two problems could be studied. The history of genetics since the rediscovery of Mendel's principles in 1900 is a history of the development of just such methods, and it is possible now to take stock of what these methods have done to improve our understanding of what has been, in spite of all research, so great a mystery—the origin of species.

This is what the present volume attempts to do. When one considers that this is 1937, a hundred years after the germ of the theory of natural selection first stirred in Darwin's mind, and nearly forty

years after Mendel's theory and methods came to recognition, one might be tempted to suppose that such a reassessment of evolution was long overdue. But a glance at the sources of Professor Dobzhansky's material shows that this is not the case. The works cited in his bibliography are largely products of the twenties and especially the thirties of the present century. This expresses very well the recency of much of our knowledge of the actual factors involved in the differentiation of species.

The reasons for this are not far to seek. Variation and heredity had first to be studied for their own sakes and genetics grew up in answer to the interest in these problems and to the need for rigorous methods for testing by experiment all ideas we might hold about them. The requirements of this search drove genetics into the laboratory, along an apparently narrow alley hedged in by culture bottles of *Drosophila* and other insects, by the breeding cages of captive rodents, and by maize and snapdragons and other plants. Biologists not native to this alley thought sometimes that those who trod along it could not or would not look over the hedge; they admitted that the alley was paved with honest intentions but at its end they thought they could see a red light and a sign "The Gene: Dead End."

That condition, if it ever existed to any marked degree, is again changing, and Professor Dobzhansky's book signalizes very clearly something which can only be called the Back-to-Nature movement. The methods learned in the laboratory are good enough now to be put to the test in the open and applied in that ultimate laboratory of biology, free nature itself. Throughout this book we are reminded that the problems of evolution are given not by academic discussion and speculation, but by the existence of the great variety of living animals and plants. The facts and relationships found in nature have to be examined from many points of view and by the aid of many different methods. Evolution, in the author's words, is a change in the genetic composition of populations, and populations follow laws which may be derived by mathematical reasoning as extrapolations of the known behavior of the fundamental units of reproduction—genes and chromosomes. It is a kind of tour de force that in this book the recent work in this field is fitted into its important place in a way which does not offend the sensibilities of those who are repelled

by mathematical formulas. Cytology too has become an essential weapon of those who would attack the species problem, and full advantage has been taken of the newer methods of studying chromosomes in Chapter four. Here too one may read a cytological detective story from the author's own experience, which, while it may shock the older makers of phylogenies, will convince the modern man that the riddle of speciation is by no means hopeless. Still other methods are brought to bear on the questions of sterility in species hybrids and of the mechanisms which are effective in keeping species separate, a field which has been recently illuminated by Professor Dobzhansky's original contributions.

In all this, the author appears not only as geneticist and as student of natural history, but as one who received his training in both fields in Russia. English-speaking biologists have special cause to be grateful for this last fact, for it has enabled Professor Dobzhansky to make available to us many important contributions from workers in the Soviet Union, where researches in this field have been actively prosecuted.

There was need for such a summary and synthesis of the new experimental evidence, and for reassessment of the older theories. It is of less importance that of these latter natural selection has survived the ordeal than that both the theory and the underlying reality, the species, have taken on new, and as one may guess, more fruitful meanings.

L. C. DUNN

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August, 1937

PREFACE

THE PROBLEM of evolution may be approached in two different ways. First, the sequence of the evolutionary events as they have actually taken place in the past history of various organisms may be traced. Second, the mechanisms that bring about evolutionary changes may be studied. The first approach deals with historical problems, and the second with physiological. The importance of genetics for a critical evaluation of theories concerning the mechanics of evolution is fairly generally recognized. The present book is devoted to a discussion of the mechanisms of species formation in terms of the known facts and theories of genetics. Some writers have contended that evolution involves more than species formation, that macro- and micro-evolutionary changes may be distinguished. This may or may not be true; such a duality of the evolutionary process is by no means established. In any case, a geneticist has no choice but to confine himself to the micro-evolutionary phenomena that lie within reach of his method, and to see how much of evolution in general can be adequately understood on this basis.

Considerations of space have forced us to refrain from a detailed discussion of some of the objections that have been advanced against the genetic treatment of evolutionary problems. Thus, Lamarckian doctrines find but a brief mention. The treatment had to be made assertive rather than polemic, dogmatic rather than apologetic.

This book is based on a series of lectures delivered at Columbia University, New York City, in October, 1936. Each lecture was followed by a discussion in which representatives of various biological disciplines took part. To these colleagues, as well as to Drs. Edgar Anderson, A. F. Blakeslee, M. Demerec, L. C. Dunn, T. H. Morgan, A. H. Sturtevant, and Sewall Wright, the writer is much indebted for many valuable suggestions and criticisms. The help of Mrs. N. P. Sivertzev-Dobzhansky in the preparation of the manuscript is gratefully acknowledged.

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VIII: ISOLATING MECHANISMS

CLASSIFICATION

THE FUNDAMENTAL importance of isolation in the evolutionary process has been recognized for a long time. Lamarck and Darwin pointed out that interbreeding of groups of individuals that are hereditarily distinct results in dissolution and swamping of the differences by crossing. The only way to preserve the differences between organisms is to prevent their interbreeding, to introduce isolation. Among Darwin's immediate followers the rôle of isolation was stressed especially by M. Wagner, in whose view it has assumed the position of keystone of the whole theory of evolution. Romanes originated the oft-quoted maxim, "without isolation or the prevention of interbreeding, organic evolution is in no case possible" which if taken too literally overshoots the mark.

From the viewpoint of present knowledge it appears that these early ideas about the rôle of isolation confused two entirely different problems. First the differences between individuals and groups may be due to a single gene or a single chromosome change. Such differences can never be swamped by crossing, since in the offspring of a hybrid segregation takes place, and the ancestral traits reappear unmodified. No isolation is needed to preserve the variation due to changes in single genes, and if one consents to dignify gene mutation by applying the name evolution, the latter is independent of isolation. The bearing of the particulate, as opposed to the blending, theory of inheritance on the problem of the retention of hereditary variation has been discussed above (Chapter V). The second class of differences between individuals and groups is genetically more complex, owing to the coöperation of two or more genes. Races and species usually differ from each other in many genes and chromosomal alterations. Species are distinct because they carry different constellations of genes. Interbreeding of races and species results in a breakdown of these systems, although the gene differences as such

are fully preserved. Hence, the maintenance of species as discrete units demands their isolation. Species formation without isolation is impossible.

On the lowest level of the evolutionary process, which is concerned with the origin of hereditary variability, with changes in the basic units such as genes and chromosomes, the rôle of isolation is naught. But on the next higher level the molding of the above elements into integrated systems takes place. The interactions of mutation pressure, selection, restriction of population size, and migration create not new genes but new genotypes, which, in the symbolic language of Wright (1932), occupy only infinitesimal fractions of the potential "field" of gene combinations. Moreover, and this is important, the part of a field occupied by a species is due not to chance alone, but corresponds to the location of one of the "adaptive peaks." Related species occupy each a separate peak, and numerous peaks in the same field may remain unoccupied, since some gene constellations have never been formed and tried out. The adaptive valleys intervening between the peaks are mostly uninhabited, and some of them are so low as to be uninhabitable.

The symbolic picture of a rugged field of gene combinations strewn with peaks and valleys helps to visualize the fact that the genotype of each species represents at least a tolerably harmonious system of genes and chromosome structures. Interbreeding of species results in the breakdown of the existing systems, and emergence of a mass of recombinations. Among the recombinations some might be as harmonious as the old gene patterns; some might be in fact better than the old ones, that is to say, new and higher adaptive peaks may be discovered. But a majority, and probably a vast majority, of the new patterns are discordant, and fall in the adaptive valleys.

We are confronted with an apparent antinomy. Isolation prevents the breakdown of the existing gene systems, and hence precludes the formation of many worthless gene combinations that are doomed to destruction. Its rôle is therefore positive. But on the other hand, isolation debars the organism from exploring greater and greater portions of the field of gene combinations, and hence decreases the chance of the discovery of new and higher adaptive peaks. Isolation is a conservative factor that slows down the evolutionary process.

The antinomy is removed if one realizes that an agent that is useful at one stage of the evolutionary process may be harmful at another stage. Gene combinations whose adaptive value has been tested by natural selection must be preserved and protected from disintegration if life is to endure. Without isolation the ravages of natural selection might be too great. But too early an isolation of the favorable gene combinations formed in the process of race differentiation would mean too extreme a specialization of the organism to the environmental conditions that may be only temporary. The end result may be extinction. Favorable conditions for a progressive evolution are created when a certain balance is struck: isolation is necessary but it must not come too early.

The mechanisms that prevent the interbreeding of groups of individuals, and consequently engender isolation, are remarkably diversified. It is an empirical fact that in different organisms, frequently even in fairly closely related ones, the isolation of species is accomplished by quite dissimilar means. Nor is it necessary that the interbreeding of a given pair of species be prevented by a single mechanism; on the contrary, one may observe that in many cases several mechanisms combine to make the isolation of two species more or less complete. It is important, however, that any agent that hinders the interbreeding of groups of individuals produces the same genetic effect, namely, it diminishes or reduces to zero the frequency of the exchange of genes between the groups. I have proposed (Dobzhansky, 1937a) the expression "isolating mechanisms" as a generic name for all such agents.

The isolating mechanisms may be divided into two large categories, the geographical and the physiological. Groups of individuals may be debarred from interbreeding by the mere fact that they live in different geographical regions, and hence never meet. Geographical isolation is believed by some investigators to be of paramount importance in the process of racial differentiation. The genetic nature of geographical races has been discussed above (Chapters III and IV). The probable rôle of the subdivision of the population of a species into semi-isolated local colonies has also been considered (Chapters V and VI). Here we may add that geographical isolation

alone is in general only a temporary measure and need not lead to a permanent segregation of the groups so isolated. Any species has a tendency to expand the area of its distribution; the forms now living in separate regions may eventually come together and meet. If no intrinsic, physiological, isolating mechanisms have developed, interbreeding will begin, and the originally separate groups will fuse together, at least in the area where the distribution regions overlap. Many examples of such a situation have been recorded, especially in plants (see Chapter X).

Geographical isolation is therefore on a different plane from any kind of physiological one. This consideration has to be qualified, because the occupation of separate areas by two species may be due not only to the fact that they have developed there, but also to the presence of physiological characteristics that make each species attached to the environment (climate, etc.) available in one but not in the other region. In this case we are dealing however with a kind of physiological isolation which is expressed in geographical terms. If two or more groups of forms are known to inhabit non-overlapping regions, no conclusions can be drawn as to the presence or absence of physiological isolation between them. Such groups, when brought together artificially or in the natural course of events, may interbreed freely or may continue to keep apart because of physiological isolation. In any concrete case only an experiment can decide what will take place. The physiological isolating mechanisms may be subdivided as follows:

I. Mechanisms that prevent the production of the hybrid zygotes, or engender such disturbances in the development that no hybrids reach the reproductive stage. "Incongruity of the parental forms" may be used as a general term for such mechanisms.*

A. The parental forms do not meet.

a. Ecological isolation—the potential parents are confined to different habitats (ecological stations) in the same general region, and therefore seldom, or never, come together, at least during the reproductive age or season.

b. Seasonal or temporal isolation—the representatives of two or more

*The writer has sometimes applied the word "incompatibility" instead of "incongruity," but unfortunately the former is used in a different sense in botanical literature.

species reach the adult stage each at a different season, or the breeding periods fall at different times of the year.

- B. The parental forms occur together, but hybridization is excluded, or the development of the hybrids is arrested.
 - a. Sexual or psychological isolation—copulation does not occur because of the lack of mutual attraction between the individuals of different species. This lack of attraction may in turn be due to differences in scents, courtship behavior, sexual recognition signs, etc.
 - b. Mechanical isolation—copulation or crossing is difficult or impossible on account of the physical incompatibilities of the reproductive organs.
 - c. The spermatozoa fail to reach the eggs or to penetrate into the eggs; in higher plants the pollen tube growth may be arrested if foreign pollen is placed on the stigma of the flower.
 - d. Inviability of the hybrids—fertilization does take place, but the hybrid zygote dies at some stage of development before it becomes a sexually mature organism.
- II. Hybrid sterility prevents the reproduction of hybrids that have reached the developmental stage at which the parents normally breed. Sterile hybrids produce either no functional gametes, or gametes that give rise to inviable zygotes. The classification of the phenomena of hybrid sterility will be discussed in Chapter IX.

A wealth of data on the occurrence of various isolating mechanisms in different subdivisions of the animal and plant kingdoms is scattered through biological literature. The genetic analysis of isolating mechanisms, with the possible exception of hybrid sterility, has however been left in abeyance. It is a fair presumption that the pessimistic attitude of some biologists (e.g., Goldschmidt, 1933c), who believe that genetics has learned a good deal about the origin of variations within a species, but next to nothing about that of the species themselves, is due to the dearth of information on the genetics of isolating mechanisms. The maintenance of the separation between species is due to the presence of physiological isolating mechanisms that hinder their free interbreeding; races of a species are as a rule not so isolated, or show only rudiments of isolation. So long as the genetics of the isolating mechanisms remains almost a terra incognita, an adequate understanding, not to say possible control, of the process of species formation is unattainable. In the following para-

graphs we shall nevertheless try to assemble some facts and to outline some suggestions that may throw light on these problems.

ECOLOGICAL AND SEASONAL ISOLATION

Data on the habitat of a species or race, as well as information on the time of year when breeding takes place, are customarily given in the systematic and ecological literature on any one group. A perusal of such literature usually reveals some examples of related species that differ in these respects. It seems clear enough that such differences may decrease the frequency, or preclude entirely, the interbreeding of the populations concerned. Investigations that are especially directed towards ascertaining to what extent the ecological and seasonal isolations are actually responsible for the maintenance of separation between species are, however, very rare. A genetic analysis of this type of difference between species or races has, to the writer's knowledge, never been made. With these qualifications in mind, we may examine a few instances in which the effectiveness of ecological or seasonal isolation suggests itself.

The experiments of Dice (1933) show that species of the mouse *Peromyscus* are as a rule not crossable under laboratory conditions, while races (subspecies) of the same species can be crossed and produce offspring. In this connection it is especially interesting that some races occur in the same general geographical region, without, however, producing intermediates or losing their distinctness. Dice (1931) has made a study of two races of *P. maniculatus* whose distribution areas overlap in a part of the state of Michigan, and found that one of them lives almost exclusively in forests and the other on lake beaches. Two other races of the same species occur together in the region of Glacier National Park, Montana; but Murie (1933) reports that one of them is confined to forests and the other to prairie habitats, and this excludes crossing. According to Pictet (1926, 1928a, b), races of the moths *Lasiocampa quercus* and *Nemophila plantaginis* occur in Switzerland at different altitudes. In the latter species the races encountered above 2,700 meters and below 1,700 meters above sea-level differ, according to Pictet, in a single gene. At 2,200 meters a hybrid population is encountered, composed

exclusively of heterozygotes; when bred in the laboratory the offspring include heterozygotes as well as both homozygotes, but in nature the homozygotes are always eliminated by natural selection. The evidence presented by Pictet is however incomplete, and his interpretations may be questioned.

The malarial mosquitoes united under the name *Anopheles maculipennis* are divided into a group of species or races that are isolated from each other, at least in part, by their habitats. A rather extensive literature is devoted to investigations of these mosquitoes. Roubaud (1920, 1932) finds in France two "biological races" of *maculipennis*, one of which preys chiefly on man and the other on domestic animals. In Holland two forms are found (de Buck and Swellengrebel 1931, de Buck, Torren, and Swellengrebel 1933), while in Italy at least four races are distinguished (Hackett, Martini, and Missiroli 1932; Missiroli, Hackett, and Martini 1933). Aside from minor differences in the morphology of the adults, the races differ in the larvae, and especially in the coloration of the eggs. All races seem to be potential carriers of the malarial Plasmodium, but only one or two of them have a preference for man's blood, or at least bite man and domestic animals indiscriminately. The geographical distribution of the latter races coincides, as might be expected, with that of the endemic malaria. The apparently well authenticated fact that the distribution of endemic malaria in Europe has contracted in historical times is supposed to be correlated with the increase in the number of domestic animals in the now malaria-free localities. Roubaud (1920) optimistically recommends, as a method of combating malaria still further, a "trophic education" of the population of *Anopheles* to train them to use animals instead of man.

The experiments of de Buck, Schoute, and Swellengrebel (1934) and others leave no doubt that the differences between the *Anopheles* races are hereditary. It is especially interesting that each race is restricted in nature to a fairly definite habitat, and does not occur elsewhere. Ecologically distinct races appear to be present also in the common mosquito, *Culex pipiens* (Weier 1935, de Buck 1935).

Seasonal isolation between two closely related species of the mollusk *Sepia* has been studied in detail by Cuénot (1933). One species

breeds in the spring in the littoral zone of the Atlantic and the Mediterranean, while the other breeds in the same localities in winter and at greater depths. Dr. Edgar Anderson kindly informs me that one of the main factors isolating certain species of *Iris* is a difference in the flowering seasons; the same is true for *Hamamelis virginiana* and *H. vernalis*. Mr. C. N. Rudkin permits me to quote his observations on the time of appearance of the adults in certain related species of butterflies in southern California, which are specifically known to occur together in the same localities (although their general distribution ranges do not coincide).

- { *Euphydryas chalcedona* (Doubleday & Hewitson)—April to June
- { *Euphydryas editha wrightii* (Gunder)—March
- { *Melitaea neumoegeni* (Skinner)—late March to early April
- { *Melitaea wrightii* (Edwards)—late April to early June
- { *Argynnis macaria* (Edwards)—late April to early June
- { *Argynnis adiaspe atossa* (Edwards)—late May to August
- { *Philotes sonorensis* (Felder)—February to mid-April
- { *Philotes battoides bernardino* (Barnes & McDunnough)—May

The exact flying times for each species are variable depending upon seasonal weather conditions and altitude, but in the localities where they occur together little or no overlapping is observed.

SEXUAL ISOLATION

An obvious prerequisite for a sexual union between individuals of the same or of different species is that the sexes meet and perform the series of acts that precede and enable fertilization to occur. In some forms this series of acts is relatively short and simple. In the oyster the chemical substance or substances that are released in water, together with the eggs and spermatozoa, stimulate other individuals within a certain range to spawn and to eject further masses of sex cells (Galtsoff 1930). In other animals the procedure is more complex. Sexual recognition marks of various kinds (specific scents, colorings, sounds, and various behavior patterns grouped under the name courtship) enable the individuals of either sex to discern potential mates. Any incongruity between the mating reactions of two groups of individuals may engender sexual isolation. The physiological basis of sexual isolation may, however, be as unlike

in different instances as are the mating reactions themselves.

Specific scents play an important role in the sex life of moths, and probably of most other insects as well. The experiments of Standfuss, Fabre, and many others have shown that if a female moth is exposed even in very artificial surroundings, males of the same species appear and try to reach the female despite the obstacles that may be placed in their way. It is established that the males sense the presence of the females at a rather great distance; the acuteness of smell so demonstrated is remarkable. Only rarely are males of species other than that to which the female belongs attracted (Federley 1932). In an experiment of the writer (unpublished), cages with females of *Dicranura vinula* and *D. erminea* were exposed on opposite sides of a house. Although in the locality where the experiment was carried out *D. vinula* was much more abundant than *D. erminea*, males of each species presently appeared near the cages that contained females of their own species. It may be noted that *D. vinula* and *D. erminea* may be crossed in captivity and produce sterile offspring (Federley 1915b).

When brought together artificially, males of a given species of moth as a rule pay no attention to females of species other than their own. Interspecific crosses may, however, be accomplished by special techniques. Both Standfuss (1896) and Federley (1929b) recommend placing a cage containing females of species A and males of B side by side with a cage containing females of B and males of A. The scent, and perhaps the sight, of females of their own species makes the males so excited that they copulate with females that they would not approach otherwise. In extreme cases copulation between distant species may be attempted even in the presence of partners of their own species.

The extent to which the mating reactions of species can differ without making the production of hybrids impossible may be seen from the work of Leiner (1934) on the fish *Gasterosteus aculeatus* and *G. pungitius*. These fish build special nests into which the females are goaded by the males; after the eggs are deposited and fertilized the female is driven away and the male stays to take care of the young. The behavior patterns of the two species are as follows:

G. pungitius

The nest is built hanging on some water plants.

The nest is composed of soft materials.

The nest has an entrance and an exit.

No preference for light or dark building materials.

The nest is not changed after the eggs are deposited.

The male swims toward the nest in zigzags, attracting the female to follow him.

The process of leading the female to the nest and the mating play coincide.

The female enters the nest with little prodding by the male.

G. aculeatus

The nest is built on the bottom, in a furrow dug by the fish.

Hard materials are used in the construction of the nest.

The nest has a single entrance.

On a light bottom dark building materials are preferred.

After egg deposition the nest is somewhat altered.

The male makes some zigzags in front of the female, and then swims straight to the nest followed by her.

A special mating play is enacted.

The male forces the female into the nest.

Leiner has obtained several hybrids from the cross *G. aculeatus* ♀ × *G. pungitius* ♂ with the aid of artificial insemination, and he gives some, though inconclusive, data that suggest that this cross takes place sometimes also in nature.

In some animals, especially among the predacious forms, hybridization is difficult because one or both of the prospective parents evince a belligerent attitude toward each other, instead of a sexual response. Wild species of sheep are very likely to kill the domestic sheep and goats offered them for mates. There exist some authentic data (Bristowe and Locket, 1929, and others) to prove that the involved courtship antics practiced by male spiders as a preliminary to copulation tend to delay the assumption by the female of an aggressive attitude. A male of a different species is simply put to death before copulation can take place. It is interesting however that in higher animals where the mating reactions involve complex systems of unconditional and conditional reflexes, large deviations from the normal behavior can be induced in experiments. Much work in this direction has been done by the Russian school of animal husbandry (see review by Serebrovsky 1935). Stallions can be trained to mount willingly a stuffed effigy of a mare and even that

of a cow, and the same is true of bulls, boars, and male sheep. Male turkeys were induced to attempt copulation with fowls. The practical application of these results is principally in the development of techniques for the collection of semen for artificial insemination. They are also interesting because they show that the bar to hybridization formed by sexual isolation can be surmounted experimentally. In general, hybrids that probably never occur in nature can be frequently obtained in properly conducted experiments.

Some experimental data on sexual isolation have accumulated in *Drosophila* literature. Sturtevant (1915, 1921) studied the process of courtship in several species of that genus, and found it to be frequently different. Sturtevant (1920-21) has also obtained indirect evidence to show that in mixed cultures *D. melanogaster* and *D. simulans* exhibit a preference for mating with partners of their own species. Similar observations have been published by Lancefield (1929) for A and B races of *D. pseudoobscura*. A quantitative study of sexual isolation has been made by Boche (unpublished). In his experiments equal numbers of freshly hatched virgin females of race A and race B of *D. pseudoobscura* were placed in the same vials with half the total number of freshly hatched males of one of the races. The males therefore were able to "choose" their mates. After about ninety-six hours all females were dissected, and the presence or absence of sperm in their seminal receptacles was determined by a microscopic examination. In every experiment males of race A fertilized more race A than race B females, and the opposite was the case for race B males. In one experiment the frequency of fertilization was determined after different time intervals: the result suggests that the males copulate first with females of their own race, but later also interracial matings occur, and finally practically all of the females are fertilized. No indication of even slight isolation between strains of the same race coming from different geographical regions was obtained.

Somewhat more extensive data were secured by the writer (unpublished) for *Drosophila pseudoobscura* and *D. miranda*. In some experiments the technique used was similar to Boche's: ten *D. pseudoobscura* and ten *D. miranda* females were confined with ten

males of one species for about ninety-six hours. The results are summarized in Table 18.

Sexual isolation between *Drosophila miranda* and *D. pseudoobscura* is undoubtedly present. Moreover, the isolation between these species is relatively stronger than that between race A and race B of *D. pseudoobscura* (see above). Indeed, a series of experiments

TABLE 18

FREQUENCY OF INTRASPECIFIC AND INTERSPECIFIC MATINGS IN MIXED CULTURES OF *Drosophila pseudoobscura* AND *D. miranda*.

MALES	<i>D. pseudoobscura</i> FEMALES FROM STRAIN	INTRASPECIFIC		INTERSPECIFIC	
		Fertilized	Unfertilized	Fertilized	Unfertilized
<i>D. pseudoobscura</i>	Seattle-6 (race B)	58	5	3	60
	Seattle-4 (race B)	54	1	1	54
	La Grande-2 (race A)	67	9	9	71
	Texas (race A)	57	2	8	52
	Oaxaca-5 (race A)	41	4	7	35
<i>D. miranda</i>	Seattle-6 (race B)	18	17	3	34
	Seattle-4 (race B)	12	31	—	43
	La Grande-2 (race A)	14	20	4	31
	Texas (race A)	11	22	—	34
	Oaxaca-5 (race A)	22	18	4	37

in which the frequency of intraspecific and interspecific matings has been determined after varying time intervals has shown that after five days practically all females of the same species as the male are impregnated. The number of interspecific matings also increases with time, but even after twenty-one days no more than 25 per cent of the females are fertilized. In a third series of experiments the technique was so modified that no choice of mates was available. *D. miranda* females were confined with an equal number of *D. pseudoobscura* males for nine days, after which the proportion of the females fertilized was determined by dissection. Different strains of *D. pseudoobscura* were used. Table 19 gives a summary of the results.

If *D. pseudoobscura* females are confined for nine days with males of their own species, practically 100 per cent of them are

fertilized. Therefore, the results shown in Table 19 give further proof of sexual isolation between the two species. In addition, these results reveal an important fact that the isolation varies in extent if different strains of *D. pseudoobscura* are used. It can be seen at a glance that males of race B display on the average a greater aversion to mating with *D. miranda* than do the males of race A.

TABLE 19

THE FREQUENCY OF FERTILIZATION (IN PER CENTS) OF *Drosophila miranda* FEMALES BY *D. pseudoobscura* MALES FROM DIFFERENT STRAINS

RACE	STRAIN	FREQUENCY	RACE	STRAIN	FREQUENCY
B	Cowichan-6	10.2±2.3	A	Pavilion-5	31.3±4.3
B	Quilcene-4	11.3±2.7	A	Lassen-1	32.7±4.6
B	Sequoia-4	17.8±2.9	A	Shuswap-3	36.4±3.8
B	Sequoia-8	29.4±3.4	A	Estes Park-1	36.9±4.2
A	Yale-7	18.3±3.0	A	Sequoia-15	40.8±3.7
A	Oaxaca-5	21.4±3.2	A	Cuernavaca-2	46.0±3.6
A	Olympic-2	23.0±2.7	A	Grand Canyon-3	50.4±4.7
A	La Grande-2	25.0±4.1	A	Julian	52.2±4.3

Within a race, especially in race A, wide differences are also observed. The degree of sexual isolation seems to stand in relation to the geographical origin of the given strain. It may be noted that the distribution area of *D. miranda* is relatively small, comprising only the territory around Puget Sound in the Pacific Northwest. This area is included in that of race B of *D. pseudoobscura*, which inhabits the Pacific Coast from southern California northward; the area of race A extends much further east and south than that of race B, but it barely comes in contact with that of *D. miranda* (on the Olympic peninsula). Strains of either race of *D. pseudoobscura* coming from localities in or near the distribution area of *D. miranda* show the greatest degree of isolation (Cowichan, Quilcene, Olympic, and Yale). Strains from somewhat more remote localities (Pavilion, Shuswap, Lassen, Estes Park) show less, and those from still more remote places (Sequoia, Julian, Grand Canyon, Cuernavaca) show least isolation. The only exception to the above geographical rule is the Oaxaca strain (from Mexico) which displays an unexpectedly high degree of isolation.

The existence of such inheritable differences between strains of a species is interesting, for the genetic mechanism determining the

isolation between species may be visualized as having arisen through a summation of intraspecific variations of this kind. The observed geographical regularity becomes doubly significant from this point of view. *Drosophila pseudoobscura* and *D. miranda* can be crossed, but the offspring produced are sterile. The occurrence of hybridization is evidently disadvantageous to the species, since it impairs the biotic potentials of both participants. Genetic factors increasing the sexual or any other isolation may therefore be favored by natural selection. The strengthening of isolation is however more immediately important for the populations of *D. pseudoobscura* that inhabit the territory close to that where *D. miranda* occurs than for the populations from more remote localities. The exceptionally strong isolation found in the Oaxaca strain (see above) may conceivably be accounted for by the fact that in Oaxaca *D. pseudoobscura* shares the same territory with another related species, *D. azteca*. The value of this conjecture is dubious however, because *D. azteca* occurs throughout Mexico, and the Mexican strains except Oaxaca do not seem to show a very great isolation from *D. miranda*.

Systematic studies on the crossabilities of different species and subspecies in the mouse *Peromyscus* have been made by Dice (1933). Ten races of *P. maniculatus*, five of *P. leucopus*, four of *P. eremicus*, and two of each *P. truei* and *P. californicus* have been tested in many combinations. The general conclusion reached by Dice is that races of a species can be crossed and produce hybrids, while the separate species (with the exception of *P. maniculatus* × *P. polionotus*) do not cross. The cause of the non-production of hybrids is not exactly known, but a sexual isolation may be suspected. It does not follow, of course, that sexual isolation may occur only between separate species and not between subdivisions thereof. Spett (1931) obtained some data that suggest the existence of a rudimentary sexual isolation between mutants of *Drosophila melanogaster*; his observations are contradicted however by those of Sturtevant (1915) and of Nikoro, Gussev, Pavlov, and Griasnov (1935).

MECHANICAL ISOLATION

The elaborate structure of the external genitalia and their accessories in many animals, especially among insects, has for a long time attracted the attention of morphologists and systematists. The

reason for this interest has been in part a pragmatic one: closely related species that are distinguishable with great difficulty by their external structures can sometimes be accurately classified by the structure of their genitalia. Ormancey (1849) seems to have been the first to apply this method for distinguishing the species of a family of beetles, and soon thereafter the method was introduced in other orders of insects, and also in spiders, mollusks, fish (the forms possessing gonopodia), mammals (especially bats and rodents), and other groups.

Although some conservative taxonomists have made vitriolic protests against the introduction of studies of genitalia as a part of the regular routine in describing species, the method has such obvious practical advantages in many genera and families that it has taken a firm root in modern taxonomy. That species are frequently easily distinguishable by their genitalia is indeed a plain observational fact; on this fact much theoretical superstructure has however been built. The great French entomologist Leon Dufour has propounded the so-called "lock-and-key" theory, according to which the female and the male genitalia of the same species (at least in insects) are so exactly fitted to each other that even slight deviations in the structure of either make copulation physically impossible. The genitalia of each species are "a lock that can be opened by one key only," hence the different species are isolated from each other simply and safely by the non-correspondence of their genitalia. In justice to Dufour it must be noted that the whole theory originated in pre-Darwinian days.

Dufour's theory has been much elaborated by K. Jordan (1905), who established an interesting contrast between geographical and non-geographical variations: variants of species living in the same locality show no correlated characteristics in the genitalia, while separate species, and sometimes geographical races of a species, do differ in the structure of these organs. Jordan adduces some further evidence in favor of the lock-and-key theory by showing that the male and female genitalia of *Papilio* species relate to each other as a positive and negative image, and proceeds to argue that geographical races become isolated from each other by variations in the genitalia, and thus become separate species. Jordan's theory is attractive

in its simplicity if we disregard his Lamarckian notions, which do not seem to constitute an integral part of it. Unfortunately, there is more evidence against it than for it.

First of all, the lock-and-key relationship between the female and male genitalia of the same species is one the whole a rather exceptional condition. In many groups (e.g., in *Drosophila*) where some species differ quite sharply in the structure of the male genitalia, the female genitalia are far more similar, and in addition are not sculptured as a negative image of the male parts. This situation seems to be common in diverse groups. To be sure, female genitalia are often as rich in specific characters as those of the males, but the specific differences may reside in the parts of the apparatus that are not immediately concerned with copulation. For example, among ladybirds (Coccinellidae) and leaf beetles (Chrysomelidae) female genitalia of related species are often distinct in the shape of the chitinous spermatheca and that of the duct uniting the spermatheca with the bursa copulatrix. Yet during copulation, the penis of the male is inserted into the bursa copulatrix but certainly does not penetrate as far as the spermatheca or its duct. Curiously enough, there is a certain correlation between the shape of the penis and that of the spermathecal duct: in some genera of the ladybirds the former has a very long appendage (flagellum), and the latter may be longer than the body length. The writer has ascertained specifically in such forms that the flagellum does not enter the duct. The external female genitalia are rather uniform in species of the same genus in Coccinellidae, contrasting with the variability of the corresponding male structures. In some families and genera, distinct species have very nearly similar genitalia; mechanical isolation can in no case be regarded as a universal method of isolation, even among insects.

The experimental evidence in favor of mechanical isolation is scanty, and is confined mostly to a single order, namely Lepidoptera. Standfuss (1896) has described crosses between species of moths where copulation leads to injuries to the female organs that result in death. Federley (1932), who is inclined to ascribe more importance to mechanical than to sexual isolation, states that the *Chaerocampa elpenor* male may copulate with a female of *Metop-*

silus porcellus (moths of the family Sphingidae), but is sometimes unable to withdraw its penis, making egg-deposition impossible. The reciprocal cross succeeds easily. Sturtevant (1921) has observed apparently successful copulation between *Drosophila melanogaster* males and *D. pseudoobscura* females, which, however, does not lead to the production of hybrid larvae or adults. Whether fertilization of the eggs takes place is unknown. Some pairs are however unable to separate, and die in copula. What causes the different outcome of this copulation is likewise unknown. No copulation between *D. pseudoobscura* males and *D. melanogaster* females has been recorded.

Against the above facts which tend to prove the effectiveness of mechanical isolation, one may set an array of observations on crosses between species with differently built genitalia which seems to cause no injury to either participant. Copulation between rather remote species is very frequently recorded in the entomological literature, although it generally remains obscure whether any offspring is produced thereby. The production of offspring is however immaterial as far as the problem of mechanical isolation is concerned, since copulation does not necessarily insure the occurrence of fertilization and development (see below). It is significant, however, that variations in body size within a species of insects have not been shown to hinder copulation. In *Drosophila* mutants, increasing and decreasing body size are known, and they can be crossed with consequent production of normal offspring (e.g., mutations giant and dwarf in *D. melanogaster*). The variations in body size due to the abundance or scarcity of food during the larval stage are likewise no impediment for copulation.

Kerkis (1931) has made a statistical study of the variability of the external characteristics and of the genitalia in the bug *Eurygaster integriceps*, and finds the latter no less variable than the former—a conclusion contradictory to the opinions of some systematists who regard the limited variability of the genitalia as an explanation of their usefulness in classification. In fact, the explanation is to be looked for in a different direction: the complexity of the structures of the genitalia is sometimes so great that the genetic differences between the species are more likely to be manifested in these structures than in the relatively simple external ones. The conjecture is corrobo-

rated by observations that show that in those genera and families where the structure of the genitalia is simple they are less useful for classification than in groups with complicated genitalia or accessory organs. It is justifiable to conclude that, although some mechanical isolation may be effective as a bar to crossing in some organisms, its significance has been exaggerated. Some systematists (e.g., Kinsey 1936) have come to the same conclusion.

The differences in the flower structure in related species of plants may prevent cross-fertilization because the flowers are pollinated by different insects. How effective this form of mechanical isolation is in nature is however obscure. That different plant families are adapted for pollination by different insects is of course well known, although some insects (e.g., the honeybee) visit a surprisingly wide range of plants. Whether species of the same genus are debarred from crossing by the same method has never been adequately studied. A perusal of the Knuth-Ainsworth Davis monograph of flower pollination (1906-09) shows that the lists of insects known to visit the flowers of related plant species are in some instances different, but it remains unclear to what extent this may be accounted for by the occupation of dissimilar ecological stations by the plants involved. Perhaps only in families with very specialized flower structures (orchids, Papilionaceae, and some others) can mechanical isolation play an important rôle.

FERTILIZATION IN SPECIES CROSSES

Copulation in animals with internal fertilization, or the release of the sexual products into the medium in forms with external fertilization, or the placing of the pollen on the stigma of the flower in plants, are followed by chains of reactions that bring about the actual union of the gametes, or fertilization proper. These reactions may be out of balance in representatives of different species, with a consequent hindrance or a complete prevention of the formation of hybrid zygotes. In animals, the processes of hybrid fertilization have been studied, for obvious technical reasons, almost exclusively in marine forms where the fertilization can be easily observed in vitro. Moreover, a majority of the experiments concern crosses between forms so remote (e.g., different orders, classes, and even

phyla), that the significance of the results from an evolutionary standpoint is limited.

Lillie (1921) has crossed two species of sea-urchins, *Strongylocentrotus purpuratus* and *S. franciscanus*. Both species inhabit the shore waters in the same locality, although *S. purpuratus* occurs between the tidemarks and slightly below the low-water mark, while *S. franciscanus* rarely lives above the low-water mark and goes to greater depths than the former. There exists consequently a partial ecological isolation between the two. Eggs of each species were placed in sea-water containing spermatozoa of the same or of the other species in different concentrations; the percentage of eggs that formed fertilization membranes and that cleaved was recorded. The concentrations of the *S. franciscanus* sperm that give from 73.3 per cent to 100 per cent of fertilization of the eggs of the same species produce from 0 to 1.5 per cent of fertilization in *S. purpuratus* eggs. With a concentration of the sperm of *S. franciscanus* that is forty times greater than is necessary to produce a 100 per cent fertilization of *S. franciscanus* eggs, only 25 per cent of *S. purpuratus* eggs are fertilized. A similar, though perhaps somewhat less pronounced, disability of *S. purpuratus* sperms to fertilize the eggs of *S. franciscanus* was also detected. Moenkhaus (1910) found in the cross between the fish *Fundulus heteroclitus* and *F. majalis* up to 50 per cent of polyspermic eggs which do not normally occur in intra-specific fertilizations. It may be noted that placing the eggs and spermatozoa in water of varying pH concentration sometimes permits the fertilization to take place where it would not do so otherwise.

The environment of the spermatozoa in the reproductive organs of the female of another species may be unsuitable for them and may cause their death, or at least a loss of fertilizing ability. Spermatozoa of higher animals are known to be highly sensitive to any variations in their environment, particularly to those in osmotic pressure. Serebrovsky (1935) gives following data for the spermatozoa of mammals as shown in Table 20.

The sperm can be preserved for artificial insemination for a long time if a proper environment is created, but the fertilizing ability is lost very quickly otherwise. The sperm of a duck, a goose, and

a cock has been injected in the genital ducts of female ducks. After 22 to 25 hours the birds were dissected, and large numbers of spermatozoa were found in the upper portions of the oviducts. But while those of the drake were alive and motile, a majority of the spermatozoa of the goose and cock were already dead (Serebrovsky 1935). Mixing the sperm of different forms may also be fatal for their

TABLE 20
MOLECULAR CONCENTRATION AND THE OSMOTIC PRESSURE OF SPERM
(after Serebrovsky)

ANIMAL	MOLECULAR CONCENTRATION	OSMOTIC PRESSURE
Man	0.297	7.5
Horse	0.302	7.6
Dog	0.319	8.1
Pig	0.335	8.4
Bull	0.335	8.4
Sheep	0.357	9.0

viability (Godlewski 1926). As far as the writer is aware, no data of a similar kind exist for crosses between closely related species.

More extensive observations of the difficulties encountered in fertilization in hybrids are available for plants. Mangelsdorf and Jones (1926) and others found that in crosses between sugary and non-sugary maize (*Zea mays*) appreciable deviations from the normal segregation ratios are obtained, the number of sugary kernels being below the expectation. Sugary differs from non-sugary in a single gene, and the results are interpreted as indicating that if a mixture of sugary and non-sugary pollen is applied to the silks of a plant containing the normal allelomorph of sugary, a competition between the pollen grains ensues, the rate of growth of sugary pollen tubes being smaller than that of the normal pollen tubes. The growth rates of the two kinds of pollen tubes on sugary silks are, however, alike. Demerec (1929b) has described an even more extreme case of incompatibility between popcorn and other varieties of maize. If popcorn is used as a female parent in crosses where non-pop pollen is applied, almost no seeds are formed. Crosses in which pop is used as a male succeed without difficulty. If a popcorn plant is double pollinated (i.e., if a mixture of pop and non-pop pollen is ap-

plied), many selfed and very few hybrid seeds are obtained. When the silks of an ear of popcorn were divided in two parts and one part was pollinated with pop and the other with non-pop pollen, the resulting ears had a full complement of seeds on the selfed side and almost no seeds on the crossed side (for further examples see Brieger 1930).

An extensive series of experiments with crosses between different species of *Datura* has been described in short preliminary communications by Buchholz, Williams, and Blakeslee (1935). They found that the speed of the pollen tube growth in the style of the same species is frequently greater than in the style of a foreign species. Species of *Datura* may differ in the length of their style, there being some correlation between the speed of the pollen tube growth and the style length. The crosses in which the species with a short style is used as the female parent and that with a long style as the male parent are in general more likely to succeed than the reciprocal crosses. Moreover, the pollen tubes may burst in the style of a foreign species before they reach the ovary, the frequency of the bursting pollen tubes being characteristic for each cross. The crossability of different species is, therefore, a function of several variables: the speed of pollen tube growth, length of the style, and the frequency of bursting pollen tubes. To this must be added also the sensitivity of the process to the environmental conditions, and the viability of the embryos (see below). The failure of the pollen grains to germinate on a foreign stigma has also been observed in some crosses.

The success of crossing of species of wheat (Watkins 1932) and of herbage grasses (Jenkin 1933) depends on several factors, one of which is the chromosome number in the parental species to be crosses. According to Watkins, the pollen tubes grow best in the styles of plants with the same chromosome number as the male parent (that is, if the ratio of the chromosome numbers in the pollen and in the style is 1:2). In the style of a species having a higher chromosomal number, the pollen tube growth is normal or reduced, while in the style with a lower chromosomal number it is much reduced. The possible rôle of self-sterility in interspecific crosses has been discussed by Anderson (1924).

The incongruity of the allopolyploid hybrids with the parental species is an exceptionally interesting fact. It may be recalled that *Raphanobrassica* is a synthetic new species obtained by a doubling of the chromosome complement in the hybrid between radish (*Raphanus sativus*) and cabbage (*Brassica*). Karpechenko (1928) and Karpechenko and Shchavinskaia (1929) have made systematic attempts to cross *Raphanobrassica* with radish, cabbage and other species of cruciferous plants. The cross *Raphanobrassica* ♀ × *Raphanus* ♂ produced only eleven seeds from 382 artificially pollinated flowers, *Raphanus* ♀ × *Raphanobrassica* ♂ eleven seeds from 143 flowers, *Raphanobrassica* ♀ × *Brassica* ♂ two seeds from 551 flowers, and *Brassica* ♀ × *Raphanobrassica* ♂ no seeds from 411 flowers. No more successful were the attempts to secure offspring from such crosses by open pollinations; when the three species are planted side by side, each of them produces almost exclusively a pure progeny. *Raphanobrassica* produces however some seeds if crossed to *Raphanus raphanistrum*, a species related to radish but crossable only with difficulty to the latter. Karpechenko believes that the incompatibility in the above crosses is due to a slow growth of the *Raphanobrassica* pollen on the *Raphanus* and *Brassica* styles, and vice versa. The pollen tube growth has however not been studied specifically, and it remains possible that inviability of the zygotes is involved. The latter has been observed in the crosses between the allotetraploid derivative of *Nicotiana rustica* × *N. paniculata* and *N. rustica* (Singleton 1932). Whatever is the mechanism, it is clear that an incongruity between an allotetraploid and its parents would be very helpful for the establishment of the former as a separate species in nature.

VIABILITY OF HYBRID ZYGOTES

The occurrence of a union between the gametes of different species gives no assurance that the zygotes so formed will produce an adult hybrid organism. As a matter of fact, the life of a hybrid zygote may be cut short at any stage, beginning with the first cleavage of the egg and up to the late embryonic or post-embryonic development. The physiology of the developmental disturbances that prevent the hybrid from reaching maturity is almost entirely unknown. The

theory that a lack of "affinity" or of "coöperation" between the ancestral germ plasms is involved gets us nowhere.

Hybridization between very remote forms (echinoderms \times molluscs, echinoderms \times annelids, etc.) frequently results in the sperm nucleus being simply eliminated from the first cleavage spindle, or else the paternal, and sometimes also some of the maternal, chromosomes are discarded in the cytoplasm and perish. Similar, although less extreme, disturbances are observed in crosses between different families and genera of sea urchins and between families and genera of amphibians (a review in Hertwig 1936). In hybrids between different fish (Moenkhaus 1910, Newman 1914, 1915, Pinney 1918, 1922, and others) all sorts of disturbances may occur, from chromosome elimination during cleavage, and arrest of gastrulation and of organ formation, to death of the advanced embryos. The above authors emphasize that the early or late death of the embryos is not necessarily correlated with the systematic remoteness or closeness of the forms crossed. In this respect the data of Zimmermann (1936) and Strasburger (1936) are very instructive. They have investigated the races of a ladybird beetle *Epilachna chrysomelina*, which inhabits southern Europe, Africa, and western Asia. This area is subdivided into several smaller regions, each inhabited by a separate race (subspecies). The crosses between most of the races that were available for experiments gave hybrids without much difficulty. But the cross between the South African form, *E. capensis*, and *E. chrysomelina* produced no larvae on account of the profound disturbances in the embryonic development. Morphologically, *E. capensis* is not much more different from *E. chrysomelina* than the races of the latter species are from each other. On the other hand, Pictet (1936) states that the viability of the hybrids between the moths *Lasiocampa quercus* from different localities is inversely proportional to the distance between the localities. Similar results have been obtained by Pictet in another moth, *Nemeophila plantaginis*, where the hybridization of local races may result in the production of unfertilized eggs (no cytological study has, however, been made).

The death of the hybrid zygotes has been observed also in plants. In some crosses between species of *Datura*, the pollen tubes reach the ovary, fertilization takes place, but nevertheless no seeds are

obtained. The hybrid *D. stramonium* \times *D. metel* develops up to the eight-cell stage of the embryo, but no further. The development of the endosperm in the same hybrid proceeds apparently normally up to the seventh day after fertilization, and then stops (Satina and Blakeslee 1935). A similar situation is encountered in crosses between some species of *Nicotiana* (McCray 1933). According to Watkins (1932), the non-production of seeds in wheat species crosses may be due to a disharmony between the development of the embryo and that of the endosperm. On account of the double fertilization process in the higher plants, the numbers of chromosomes in the embryonic and in the endosperm tissues are normally as 2:3. If a species with a high chromosome number is used as the pollen parent and that with a low number as the mother, the ratio of the chromosome numbers in the embryo and the endosperm is $> 2:3$, and the embryo dies. The reciprocal cross, giving rise to a ratio $< 2:3$, is less deleterious for the viability of the hybrid.

In some instances the constitutional weakness of the hybrid organism entails no great disturbances in the fundamental life processes, and the application of certain treatments enables the experimenter to bring to maturity hybrids that do not survive otherwise. A remarkable example of this phenomenon is afforded by the work of Laibach (1925) on hybrids between species of flax. In the cross *Linum perenne* \times *L. alpinum*, the hybrid seeds are able to germinate with some difficulty. The seeds from the cross *L. perenne* \times *L. austriacum* fail to germinate if left to their own devices. If, however, the embryos are artificially freed from the seed coat (the seed coat being here a purely maternal tissue), germination does take place, and the young seedlings may give rise to luxuriant hybrid plants that are fertile and produce normal seeds of the F_2 generation. Still greater is the suppression of the seed development in the cross *L. austriacum* \times *L. perenne*, and yet it can also be surmounted. The diminutive embryos are extracted from the seeds and placed in a nutrient solution containing from 10 per cent to 20 per cent sugar, where they continue to grow; after some days they are transferred to moist filter paper, and allowed to germinate. The seedlings are then planted in soil.

In crosses between species of certain moths only males appear

among the adult hybrids (*Chaerocampa elpenor* ♀ × *Metopsilus porcellus* ♂ and *Deilephila euphorbiae* ♀ × *D. galii* ♂); females are present among the caterpillars but they die in the pupal stage. The reciprocal crosses give hybrids of both sexes (Federley 1929). Bytinski-Salz (1933) implanted the ovaries of the pupae that normally die into the pupae of the parental species. The implants developed in the new host far beyond the stage at which they would die in the body whence they came, thus proving that the inviability of a hybrid as a whole need not extend to all its tissues.

The appearance of unisexual progenies recorded in the crosses just discussed is a fairly common phenomenon in interspecific hybrids in animals; individuals of one sex die, while the viability of the other sex is affected little or not at all. Haldane (1922) has formulated a rule that, with some exceptions, holds rather well: "when in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex." In mammals, Amphibia, and most insects, males are known to be heterozygous (XY) and females homozygous (XX) for sex, and accordingly male hybrids are defective more frequently than females. On the contrary, in birds, butterflies, and moths, females are heterozygous (XY) and males homozygous (XX); here female hybrids tend to be less viable than males.

A possible mechanism that may underlie Haldane's rule was suggested by Dobzhansky (1937b). It is known (Chapter IV) that *Drosophila pseudoobscura* and *D. miranda* differ in gene arrangement, and, what is especially important for us now, some genes that lie in one of these species in the X chromosome lie in the other in the autosomes, and vice versa. The cross *D. miranda* ♀ × *D. pseudoobscura* ♂ produces fairly viable female and abnormal male hybrids; the reciprocal cross gives rise to viable females, but the males die off. Suppose that *D. pseudoobscura* has in its X chromosome a certain group of genes *A* that lie in the autosomes of *D. miranda*, and that a group of genes *B* which in *D. miranda* lies in the X chromosome is located in the autosomes of *D. pseudoobscura*; with respect to these genes, the constitution of the females of both species and of the female hybrids is alike, namely *AABB*. Males of *D. pseudoobscura* and the male hybrids from the cross *D. miranda* ♀ × *D.*

pseudoobscura ♂ are *ABB*; *D. miranda* males and the male offspring from the cross *D. pseudoobscura* ♀ × *D. miranda* ♂ are *AAB*. The genotypes of the pure species are evidently so adjusted by countless generations of natural selection that the constitution *ABB* in *D. pseudoobscura* and *AAB* in *D. miranda* permits the development of the "normal" males of the respective species. But the genotype of a hybrid is on the whole a compromise, an intermediate, between those of the parental species; the constitution *AABB* is normal for females of either parent and for the hybrid females as well. The constitution *ABB* is however incompatible with the genotype of *D. miranda*, and *AAB* with that of *D. pseudoobscura*. The hybrid males suffer from a disturbance of the genic balance, and consequently have an impaired viability. An explanation of this type is applicable only to hybrids between species that differ in the distribution of genes among the X chromosome and the autosomes; this is known to be the case for *D. pseudoobscura* and *D. miranda*, but in other species crosses, critical data are lacking. The explanation can be made more general if one assumes that many species have a balance of genes in the X chromosome and the autosomes peculiar to themselves and different from other species. This balance may remain undisturbed in the homozygous sex in the hybrids, but it is likely to be upset in the heterozygous sex.

From a geneticist's point of view, it is especially important that the viability of the hybrids between the same two species may depend on the particular strains of the parental species used in the cross. Such facts may throw some light on the mechanism of the origin of isolation. The outcome of the cross *Crepis capillaris* × *C. tectorum* is variable; in some cultures all the hybrid seedlings die in the cotyledon stage, in others only half of the seedlings die, and in still others the hybrids are viable. Hollingshead (1930a) has shown that certain strains of *C. tectorum* carry a dominant gene which in the pure species produces no visible effects, and in particular has no apparent influence on the germination of the seedlings. If, however, a hybrid between *tectorum* and *capillaris* carries this gene, it does not develop beyond the cotyledon stage. The crosses in which the *tectorum* parent is homozygous for the gene in question produce accordingly no viable seedlings, while 50 per cent or

100 per cent of such seedlings occur in cultures in which the gene is heterozygous or absent respectively. Further experiments have shown that the same gene is lethal for the seedlings of the hybrids *C. tectorum* × *C. leontodontoides* and *C. tectorum* × *C. bursifolia*, but not in the crosses *C. tectorum* × *C. setosa* and *C. tectorum* × *C. taraxacifolia*. It may be noted that the wild populations of *C. tectorum* from some localities carry the gene, and those from other localities are free from it.

An analogous situation has been observed in crosses between the fish *Platypoecilus maculatus* and *Xiphophorus helleri* by Bellamy (1922), Kosswig (1929), and others. The dominant sex-linked gene *N* causes in *Platypoecilus* an increase of the black pigmentation compared to the recessive condition. If a *Platypoecilus* carrying *N* is crossed to *Xiphophorus*, *F*₁ hybrids are obtained that are heterozygous for *N*, but which show a greater extension of the black pigment than in the case of either heterozygous (*Nn*) or homozygous (*NN*) *Platypoecilus*. A backcross of the *F*₁ individuals (*Nn*) to *Xiphophorus* (*nn*) gives some heterozygotes (*Nn*) with a pathologically over-developed black pigmentation, which results in the appearance of melanotic tumors. The gene *N* is therefore innocuous for viability on the genetic background of *Platypoecilus*, but becomes virtually a lethal when introduced into the genotype of *Xiphophorus*. The exaggeration of the unfavorable effects of certain genes of *Drosophila pseudoobscura* in the hybrids between race A and race B of this species will be discussed below (Chapter IX). According to Kostoff (1936), the cross *Nicotiana rustica* var. *humilis* × *N. glauca* gives hybrids that die as early embryos, while in the *N. rustica texana* × *N. glauca* viable hybrids are obtained. According to Backhouse (1916) and Meister and Tjumjakoff (1928), success in the crosses between wheat and rye depends on the varieties of the parental species used.

THE ORIGIN OF ISOLATION

Despite the appallingly insufficient attention that the problem of isolation has received in genetics, there is every reason to believe that a great variety of isolating mechanisms are at work in nature, preventing the exchange of genes between populations of different

species. The mode of origin of these mechanisms remains a puzzle, however, and some writers (e.g., Bonnier, 1924, 1927) are inclined to believe that the known genetic principles are insufficient to account for it. The scheme that is outlined in the following paragraphs is to be taken as a working hypothesis that may or may not prove useful in further work.

It is indeed difficult to conceive how isolation between two groups of individuals might arise through a single mutation. Mutations that change the sexual instincts, or the structure of the genitalia, or the physiology of the gametes, or some other properties of their carriers that are essential for reproduction may occur. Such mutations may prevent crossbreeding of the modified and the ancestral types, but this is not yet sufficient to produce a workable isolating mechanism. For isolation encountered in nature has always two aspects: the crossing of individuals of group A with those of group B is made difficult or impossible, but individuals of A as well as of B are fully able to breed *inter se*. A mutation that would produce isolation must therefore not only prevent crossbreeding between the mutant and the original type, but must simultaneously insure the normal crossability of the individuals carrying the mutation. In other words, it is essential not only that interbreeding between A and B be debarred, but also that a new and harmonious system of physiological reactions arise that would allow the propagation of the new type. Such a coincidence can hardly be imagined to be a common occurrence.

This difficulty does not apply to the origin of ecological or seasonal isolations, since these isolating mechanisms do not necessarily involve a reconstruction of the morphology or physiology of the reproductive system. However, unless the species concerned is capable of self-fertilization, even here the origin of isolation through a single mutational step is rather unlikely, since the mutant can hardly become established in nature. Let us suppose, for example, that the ancestral form and a mutant reach sexual maturity at different seasons, or exist in different ecological niches. With mutation rates that are as low as those observed for most genes in the laboratory, the number of mutants produced in each generation would be so small that they could hardly find mates. Only where a partial sea-

sonal or ecological isolation obtains can their origin by a single mutation be envisaged.*

It is more probable that the formation of isolating mechanisms entails building up of systems of complementary genes. Let us assume that the ancestral population from which two new species are evolved has a genetic constitution $aabb$, where a and b are single genes or groups of genes. Assume further that this ancestral population is broken up into two parts that are temporarily isolated from each other by secular causes, such as a geographical isolation. In one part of the population, a mutates to A and a local race $AAbb$ is formed; in the other part, b mutates to B , giving rise to a local race $aaBB$. The individuals of the constitutions $aabb$, $Aabb$, and $AAbb$ are able to interbreed freely with each other, hence there is no difficulty in the gene A becoming established in the population; the same is true for $aabb$, $aabb$, and $aaBB$ individuals. But the cross $AAbb \times aaBB$ is difficult or impossible, because the interaction of A and B produces one of the physiological isolating mechanisms discussed above. It follows that when carriers of the genotypes $AAbb$ and $aaBB$ come again in contact (because they have surmounted the geographical boundaries that have separated them, for example, or due to a change in their environment), they will be prevented from interbreeding by physiological causes.

The scheme just outlined may appear fanciful; it is useful, however, to examine it further to see whether the assumptions it involves are justified by factual data. One of the basic postulates is that the development of physiological isolating mechanisms is preceded by a geographical isolation of parts of the original population. The observational studies on variation in nature furnish a good deal of evidence to support this thesis. Since Darwin, and especially since Wagner, it is regarded as probable that the formation of geographical races is an antecedent of species formation; more recently this

* The origin of allopolyploids accompanied by isolation from the ancestral species may appear to vitiate the above arguments. This is not the case. Allopolyploids may be produced *en masse* in the localities where the distributions of the ancestral species overlap, and furthermore only those allopolyploids may be established in nature which happen to be isolated from their ancestors. The reduplication of the chromosome complement induces an isolation automatically, since a cross tetraploid \times diploid gives rise to triploid offspring which is always unstable if propagated sexually. In general, the effects of polyploidy could hardly be likened in this respect to those of gene mutations.

dictrine has been strongly supported by many investigators, among whom we may name K. Jordan (1905), D. Jordan (1905), Semenov-Tian-Shansky (1910), Rensch (1929), and Kinsey (1936). Some systematists regard it as one of the greatest generalizations that has resulted from their work. The distribution regions of races of the same species as a rule do not overlap, while the areas of separate species frequently do. Now, coexistence of distinct groups of individuals in some locality without formation of intermediates and of recombinations of characters is indirect evidence that these groups are isolated physiologically (provided, of course, the differences between them are due to more than one gene). The assumption that geographical isolation is a *conditio sine qua non* of species formation is, nevertheless, not a necessary one. We have seen that ecological isolation may conceivably arise from a single mutation, and it may enable the groups of individuals to develop other physiological mechanisms. All that is necessary for the development of the latter is that some kind of isolation is present to start with. Once an isolating mechanism has appeared, the formation of additional mechanisms to strengthen the action of the first is made much easier. The interbreeding of two species is frequently prevented not by a single but by several mechanisms reinforcing each other's action (Dobzhansky 1937a). The question which of these mechanisms has developed first can be answered only by conjectures, however.

A geographical isolation of parts of a population may be followed by the appearance in the subgroups of inheritable changes that engender a permanent isolation between them. It follows that we may witness in nature isolating mechanisms *in statu nascendi*, when some individuals are already isolated and others not yet isolated from other species. The experiments of Hollingshead (1930a) discussed above furnish an admirable example of such a situation. A part of the population of *Crepis tectorum* is still able to produce viable offspring when crossed to *C. capillaris* and to certain other species, while other individuals produce only inviable hybrids. The gene responsible for the death of the hybrids has not yet permeated the entire population of *C. tectorum*. The variable intensity of sexual isolation between *Drosophila pseudoobscura* and *D. miranda* (see above) presents the same picture. Further experiments may be expected to uncover many new instances of this kind.

The spread within a population of genes that may eventually induce isolation between populations is probably due to their properties other than those concerned with isolation. What these properties are is a moot question, and here is the weakest point of the whole theory. What, for example, is the rôle played within *Crepis tectorum* by the gene that causes the death of the hybrids with *C. capillaris*? Hollingshead was unable to detect any effects of this gene, except that manifested in the hybrids. Isolation is in general a concomitant of the genetic differentiation of separate populations. It may be noted, however, that only those genetically distinct types that have developed isolation can subsequently coexist in the same region without a breakdown of the differences between them due to crossing. Therefore, isolation becomes advantageous for species whose distributions overlap, provided that each species represents a more harmonious genetic system than the hybrids between them. Under these conditions the genes that produce or strengthen isolation become advantageous on that ground alone, and may be favored by natural selection. This may be at least a partial solution of the difficulty stressed above.

Lastly, one may inquire whether the genes that within a species are harmless or even useful can become deleterious in combination with other genes in a hybrid. The inviability (and, in part, the sterility) of hybrids appears to be due to such an action of genes, each of which taken separately produces no disturbance in its carrier. The observations of Hollingshead on *Crepis* and of Bellamy and Kosswig on *Platyopocilus* and *Xiphophorus* furnish incontrovertible evidence in favor of this assumption. The appearance of "novel" characters in hybrids is indeed one of the well known genetic phenomena. In this respect the recent observations of Irwin and Cole (1936) and Irwin, Cole, and Gordon (1936) on doves and pigeons are very suggestive. By means of immunogenetic reactions these investigators have detected in the blood certain species-specific substances. The hybrids have however not only the substances characteristic for their parents, but also certain "hybrid" substances that are absent in the parental species. The amount of hemoglobin in the blood of the hybrids between the yak and domestic cattle exceeds that present in either parent (Kosharin and Samochwalowa 1933).

IX: HYBRID STERILITY

INTRODUCTION

THE PROBLEM of hybrid sterility goes back at least to Aristotle, who in the "De generatione animalium" discussed at length the sterility of the mule. Aristotle's explanation of the sterility of mules has only an historical interest. In recent times much work has been done, and many valuable observations collected on sterile hybrids in various animals and plants. The phenomenology of hybrid sterility is now fairly well known, but its causal analysis is confronted with difficulties which have been only partly overcome. The time for a synthetic treatment of the subject is probably not yet at hand.

Sterile hybrids are frequently vigorous somatically, but their reproductive organs, more precisely the gonads, show derangements that prevent the development of functional sex-cells. This contrast is characteristic of sterile hybrids. The mule appears to have as harmoniously organized a system as either of its parents; in fact, under some conditions mules are superior in viability to the parental species. And yet, the testes and the ovaries of mules are manifestly abnormal, no spermatozoa or mature eggs being formed in them. The reduction of the viability and the sterility of hybrids are distinct phenomena. One might perhaps object to making such a distinction on the ground that the gonads are the place of least resistance, and their deterioration is a sign of some general weakness of the whole organism. This objection is invalid; a constitutional weakness in pure species is by no means always accompanied by sterility, and many hybrids with reduced viability are not sterile (e.g., the hybrids between the flax species described by Laibach, cf. Chapter VIII).

A dissociation between the processes taking place in the gonads of a hybrid and those in its soma has been observed in *Drosophila pseudoobscura* by Dobzhansky and Beadle (1936). The male hy-