

PRESIDENTIAL ADDRESS

A Case History of Evolution

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This is the 75th annual meeting of the Indiana Academy of Science. The first meeting was held in Indianapolis in 1885 and it is fitting that this one should also be held here. It is indeed gracious of Butler University to place its facilities at the disposal of the Academy for this anniversary occasion and we are much in the debt of President Ross and those at the university who have worked so hard to make this meeting an outstanding one, especially to Dr. Wm. Bessey, chairman of the Program Committee, and his able associates.

The first president of the Academy was David Starr Jordan and the second was John M. Coulter. There were giants in those days. Both men were noted students of evolution, the one in animals, the other in plants. It would not be inappropriate, therefore, on this occasion to center our discussion around the topic of evolution, especially as it happens that for scientists, and especially for life scientists, 1959 is a very special anniversary year, the centenary of the publication of Chas. Darwin's *Origin of Species*. The appearance of this book is perhaps the most significant single event that has transpired in the history of modern science, for it revolutionized scientific thinking, not only in biology, but in the other sciences as well, and gave to post-Darwinian science a character, a point of departure, radically different from that of the pre-Darwinian period. Darwin's great contribution was that he brought forward convincing evidence in support of what had been prior to this time an unproved hypothesis in the minds of a handful of men. Darwin's contribution not only convinced biologists in general of the fact of organic evolution, it started scientists in other fields thinking in evolutionary terms. Intrigued with the thought of progressive evolution, scientists began to apply evolutionary thinking to the question of origins in all fields—the origin of the elements, the earth, the planets, the stars and galaxies; and the universe of which we are a part began to take on added intelligibility when interpreted as the result of a long and continuous process, involving the operation of physical and chemical laws in a universe of matter. Sciences before and after Darwin were very different disciplines, differently oriented, differently pursued.

It is therefore rather surprising to realize that, although Darwin's work has had such a profound influence in persuading scientists of the reality of evolution, very few cases have actually been found in which the forces that have operated to bring about specific evolutionary changes have been identified and the changes analyzed in detail. The organisms that have been studied with sufficient thoroughness so that we know not only what steps have taken place in the course of evolution, but also what the nature and relative importance of the factors bringing about these changes have been are few indeed. There are a good many cases where evolutionary changes can be traced, as in the horse or elephant, but the causal factors that brought these transformations about are either unknown or a matter of speculation.

I wish this evening to give in brief outline an account of one organism

in which some of the causal factors are becoming understood. This is probably a fitting subject for discussion in connection with the anniversary of both the Indiana Academy of Science and the Origin of Species.

The organism in question is the evening primrose, *Oenothera*. This is a large genus, native only to the Western Hemisphere, though now widespread in other parts of the world. It consists of 15 subgenera, only one of which, the largest, and the one that has been most studied, will be discussed here (*Euoenothera*).

Curiously enough, it was in connection with evolutionary studies that *Oenothera* first came into scientific prominence. de Vries used it as the principal basis upon which he founded his celebrated and influential Mutation Theory of Evolution. It turned out, however, that the phenomena which he observed in *Oenothera* and which led him to this theory were not what he thought they were, and did not support his theory. In consequence, *Oenothera* lost for a time its central evolutionary interest. It continued, however, to be of cardinal importance to biologists because of its peculiar hereditary behavior, analyzed so successfully by Renner and his students. It later became a center of cytological and cytogenetic interest because of the peculiar behavior of its chromosomes at the time that reproductive cells were formed, a behavior that accounted satisfactorily for its unusual hereditary behavior. In recent years, however, it has again become the subject of intense evolutionary study, for it turns out that one can trace evolutionary developments and determine relationships between the various populations by utilizing clues afforded by its cytogenetic behavior.

In order to understand the nature of the evolutionary factors operating in this genus, it will be necessary briefly to explain the peculiarities in cytological and genetical behavior which *Oenothera* displays. *Oenotheras* have 14 chromosomes. Instead, however, of these chromosomes arranging themselves in pairs at the formation of reproductive cells, as they do in other plants and animals, they are arranged end to end to form a closed circle (fig. 1A). At the time of separation of these chromosomes into the



Fig. 1. Chromosomes of *Oenothera*, arranged in a closed circle of 14. A, sometime prior to separation. B, just before separation of adjacent chromosomes to different cells.

daughter cells, the chain becomes aligned across the cell (fig. 1B), and adjacent chromosomes are separated to opposite ends of the cell, and hence into different reproductive cells. It turns out that chromosomes of paternal and maternal origin alternate in the chain (fig. 2); consequently, separation of adjacent chromosomes means that all seven chromosomes



Fig. 2. Diagram showing that chromosomes of paternal and maternal origin are separated into different reproductive cells.

of paternal origin go to one daughter cell, all seven maternally derived chromosomes go to the other. As a result, only two kinds of reproductive cells are produced, genetically speaking, and these are genetically identical with the germ cells that united to form the plant.

One would expect that, since only two kinds of egg and two kinds of sperm are formed, self pollination would result in three kinds of offspring, one quarter receiving the *a* set of chromosomes from both sperm and egg, one quarter receiving *b* sets from both, and one half receiving the *a* set from one and the *b* set from the other. This does not happen. Only individuals receiving one *a* set and one *b* set are formed (fig. 3). This is

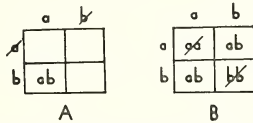


Fig. 3. Diagrams to show action of lethals. A. Lethals that kill gametes. The paternal set of chromosomes (*a*) has a lethal that kills all eggs receiving this set. The maternal set (*b*) has a lethal that inhibits sperms that receive this set. B. Lethals that kill organisms which receive this lethal through both sperm and egg.

because each set of chromosomes contains what is known as a lethal which makes it impossible for an individual to receive the same set through both sperm and egg. Consequently, a plant when selfed produces only progeny that are identical with itself genetically. To cap the climax, it turns out that practically all *Oenothera*s with circles of 14 are by nature self-pollinating. Consequently they form true-breeding lines or races, more or less isolated from each other reproductively because they ordinarily do not experience cross pollination. The population as a whole consists of a multitude of such races, in each of which all individuals in all generations are as a rule genetically identical (fig. 4).

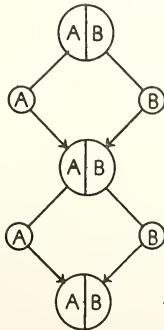


Fig. 4. Diagram to show that an *Oenothera* plant produces only progeny like itself, and consequently breeds true.

One curious result of this situation is that single sets of genes (the *a* set and the *b* set, for instance) are carried on intact from generation to generation. The union of all chromosomes into a single circle, the separation of adjacent chromosomes into different germ cells, and the presence of lethals in each set of genes, coupled with self pollination, mean that a given set of chromosomes with its genes is an entity of indefinite duration. Renner called such continuing sets of genes "complexes" and they are now known as "Renner complexes." A race with a circle of 14 and balanced lethals (i.e., a lethal in each complex) is called by Renner a "complex-heterozygote" because it is heterozygous or hybrid in that it has 2 different complexes and is prevented by the lethals from having a complex in double dose. Each race has its own complexes and different races have, as a rule, different complexes for which they are heterozygous.

Why do the chromosomes arrange themselves end to end in closed circles instead of pairs? This has been found to be due to a process known as reciprocal translocation. Suppose we postulate a race with only paired chromosomes. There will be two chromosomes of each kind and when germ cells are in the process of formation, each chromosome will seek out the one that corresponds with it and will pair with it by a process known as synapsis, which is a mysterious attractive force that develops between corresponding chromosomes during germ cell formation. Let us give numbers to the ends of a couple of pairs, 1 and 2 for one pair, 3 and 4 for

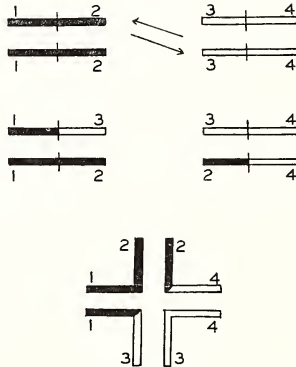


Fig. 5. Diagrams to show how an exchange between non corresponding chromosomes will result in a circle of 4 chromosomes.

another (fig. 5). Now suppose that an exchange of segments occurs between two non-corresponding chromosomes. The unchanged chromosomes will have ends 1 and 2, 3 and 4 but the changed chromosomes will have ends 1 and 3, 2 and 4. One germ cell will get 1.3 and 2.4 and the other 1.2 and 3.4. When a plant which has received 1.2, 3.4 from one parent and 1.3, 2.4 from another forms germ cells, synapsis between corresponding parts of chromosomes will produce a circle of 4 chromosomes instead of two pairs. By successive interchanges, plants with larger and larger circles can be formed.

In other words, sequences of reciprocal translocations result in a shuffling of the end segments of chromosomes, and the various complexes in the various races have come to have various associations of end segments by such a shuffling process, each complex, however, having always

the same arrangement. There are 135,135 possible arrangements of 14 ends in pairs to form the 7 chromosomes of a set or complex. No one knows how many arrangements exist in nature, but the tiny sample of races that we have analyzed has yielded over 160 different arrangements, and there are no doubt many hundreds, if not thousands, of arrangements in existence.

If two complexes differ by one interchange they will form a circle of 4 and 5 pairs when combined in a single plant. If they differ by two interchanges, they will give when combined two circles of 4 or a circle of 6. If they differ with respect to all their chromosomes they will in most cases produce a circle of 14. It is amazing that in almost every race from the Rockies eastward the associated complexes differ so greatly from each other that they yield a circle of 14.

Some studies made 30-35 years ago suggested strongly that the more closely complexes are related genetically the more alike will they be in segmental arrangement, and vice-versa, and subsequent studies have tended to confirm this suggestion. This means that the complexes found in most wild races are genetically unrelated to each other. On the other hand, complexes in different races are often closely related.

This summary will give us a glimpse of the unusual chromosome behavior in *Oenothera*, which results in peculiar genetic behavior. It will also help us realize that the *Oenothera* population is peculiarly constructed, being composed of a welter of true-breeding races, each isolated from the others reproductively by its self-pollinating habit, each composed of two unrelated complexes, which complexes, however, are closely related oftentimes to complexes in other races. What have been the factors that have brought to pass this unusual situation?

Without going into detail in regard to the evidence, for which there is no time, I can outline the situation briefly, as it appears to us at present.

In the first place, the evolution of this peculiar situation could not have come about if the ancestral *oenotheras* had not had a particular type of chromosome structure to start with. The ancestral forms seem to have had chromosomes with the following characteristics: (a) they all had the same size and shape; (b) they all had two equal arms, the part of the chromosome that actively moves toward one end of the cell or the other (the so-called centromere) being in the middle of the chromosome; (c) the chromosomes were all more fragile in the immediate region of the centromere than elsewhere, so that when breaks occurred, these breaks were more likely to occur at the centromere than elsewhere. We shall see shortly why these facts are important.

The second important factor in the evolution of the *oenotheras* is the occurrence and retention of translocations or exchanges of segments between non-corresponding chromosomes. While such exchanges are known in other organisms, there is no other organism where they have occurred as frequently, or at least have survived as frequently as in *Oenothera*. The reason for the frequency of survival of exchange chromosomes in *Oenothera* is to be found in the fact that exchange chromosomes have not been altered structurally. Because breaks occur at the centers of equal-sized chromosomes, exchanged segments are equal, the centromeres in the resulting circle are equally spaced, the forces are uniformly distributed

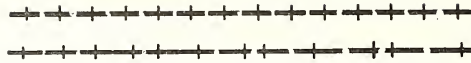


Fig. 6. (Upper line). Chromosomes are all of equal size, have median centromeres which are thus equally spaced in the circle. (Lower line). Chromosomes are unequal in size and length of arm, and centromeres are therefore unequally spaced.

and the chromosomes separate with a high degree of regularity (fig. 6, upper line). In other organisms, chromosomes are often of differing size and shape, and the exchanges of segments may be unequal between chromosomes with unlike structure. As a result, when a circle is formed, the centromeres will be unevenly spaced around the circle. Some of them may be close together, others farther apart (fig. 6, lower line). In consequence, the forces operating at the centromeres to move the chromosomes to opposite ends of the cell are unevenly spaced and a regular separation of adjacent chromosomes does not uniformly occur. Adjacent chromosomes often go into the same germ cell instead of different ones. This means that a daughter cell gets some genes twice and fails to get other genes at all. The germ cells resulting from such irregular separations fail to develop because they lack certain genes, and sterility results. In *Oenothera* this occurs only exceptionally.

In most organisms, reciprocal translocation must be classed as a harmful phenomenon because it leads to irregular distribution of chromosomes to the reproductive cells and hence results in sterility. In such organisms, a translocated chromosome tends to get weeded out because the germ cells getting it are often sterilized. The only cases where translocations between unequal chromosomes have managed to survive are in plants that are able to propagate vegetatively and hence are more or less independent of the sexual process. In *Oenothera*, however, translocations have not proved deleterious, because they do not alter chromosome structure and hence do not bring about the unequal distribution of forces that cause failure of regular separation of paternal and maternal chromosomes.

Translocations, then, are the second factor of evolutionary importance in *Oenothera*. They are important because they have made the large circles possible.

The third factor of evolutionary importance in *Oenothera* is the appearance and retention of lethals. It is the lethals that ensure that the races will breed true and that they will be maintained in a heterozygous condition, i.e., with two different complexes in each plant. Lethals in most organisms are highly deleterious. They kill, either the germ cells in which they come to lie or the embryos which receive the same lethal from both parents. If a lethal is the kind that kills eggs, it will kill half of all the eggs produced since half the eggs will have this particular gene. If it kills sperms, half the sperms will be inactivated. If so-called zygote lethals are present (i.e., lethals that kill embryos which receive them twice), and the plant is selfed, each lethal will kill one quarter of the progeny; and if both complexes have such a lethal, one half of all the progeny will be killed by the lethals. *Oenothera* is no exception to this. The lethals do kill, in *Oenothera* as in other organisms.

In *Oenothera*, however, the lethals have tended to survive in races that have circle 14, and not to be weeded out, because they have compensating advantages. These advantages stem from two facts: (1) lethals,

when present in a large circle, preserve the heterozygosity (the hybridity) of all the chromosomes in that circle, and thus ensure and maintain maximum hybrid vigor. They do so at the cost of 50% sterility of the germ cells or embryos, but those plants that are produced are unusually well equipped for the struggle for existence. Maximum hybrid vigor gives them a high level of survival value. (2) Balanced lethals in a circle of 14 help to make the race true breeding. If it is a vigorous, successful race, all individuals in the race, in all generations, will show this vigor and this success.

Lethals in paired chromosomes do not afford sufficient advantage to compensate for their killing effect. A lethal in a chromosome that forms a pair with its corresponding chromosome will kill 50% of sperms or eggs, or one quarter of the embryos, just as will the lethal found in a circle of 14, but it will not produce true breeding, and it will preserve the heterozygosity of one chromosome only, which is not enough of an advantage to compensate for its bad effect. True breeding and maximum hybrid vigor are found only when all chromosomes are included in a single circle. This is no doubt why almost all races from the Rockies eastward have a circle of 14. Only those with a circle of 14 have survived.

A fourth factor of importance is the development of a self-pollinating habit. Self pollination is ordinarily a harmful practice, for it results in close inbreeding with consequent loss of hybrid vigor and general deterioration. In the case of the evening primrose, however, this cannot occur, for the lethals present in a circle of 14 ensure the maintenance of heterozygosity of all the chromosomes, and by preventing homozygosity prevent the weakening that would ordinarily come about through inbreeding. On the other hand, inbreeding confers a benefit in *Oenothera*, in that it helps to overcome the sterilizing effect of the lethals. The flowers of the evening primrose do not have to depend on the chance visits of insects, bringing sometimes abundant, but sometimes scanty, supplies of pollen. Self pollination ensures that, in every healthy flower, the stigma will be covered with pollen and as a result every viable egg will be fertilized. Since self-pollination occurs many hours before the flower opens, the eggs are being fertilized, or have already been fertilized by the time insects reach the flower with foreign pollen.

We have, therefore, a number of factors which, taken together, make for true-breeding, vigorous, highly prolific plants. Equal-sized and equal-armed chromosomes, more easily broken at the center than elsewhere, make it possible for translocations to survive. Translocations, lethals, and self pollination are all deleterious alone, but taken together each overcomes the bad effects of the others and the combination of the three is highly advantageous to the plant. One may conclude that apparently deleterious changes do not always have a bad effect, are not always to be considered retrogressive in nature.

But how have the large circles come about? Have they gradually developed as the result of successive translocations, small circles gradually becoming larger and larger as interchange has been piled on interchange? The evidence seems to indicate that this is not how the large circles have come about. A fifth factor has proved to be of evolutionary importance in the genus, namely hybridization. Large circles have been produced by

hybridization. In order to understand the role of this factor, it is necessary to explain briefly how the facts to be presented have been obtained.

Assuming as a working hypothesis that complexes whose chromosomes have similar arrangements of ends are closely related, whereas those with dissimilar arrangements are relatively unrelated, we have attempted to analyze the arrangements of ends in as many complexes as possible in terms of a standard complex. We chose as our standard one that seemed to us on various grounds primitive and possibly possessed of the original end arrangement. By a process of reasoning too complicated to go into here, we have determined the arrangement of ends in more than 360

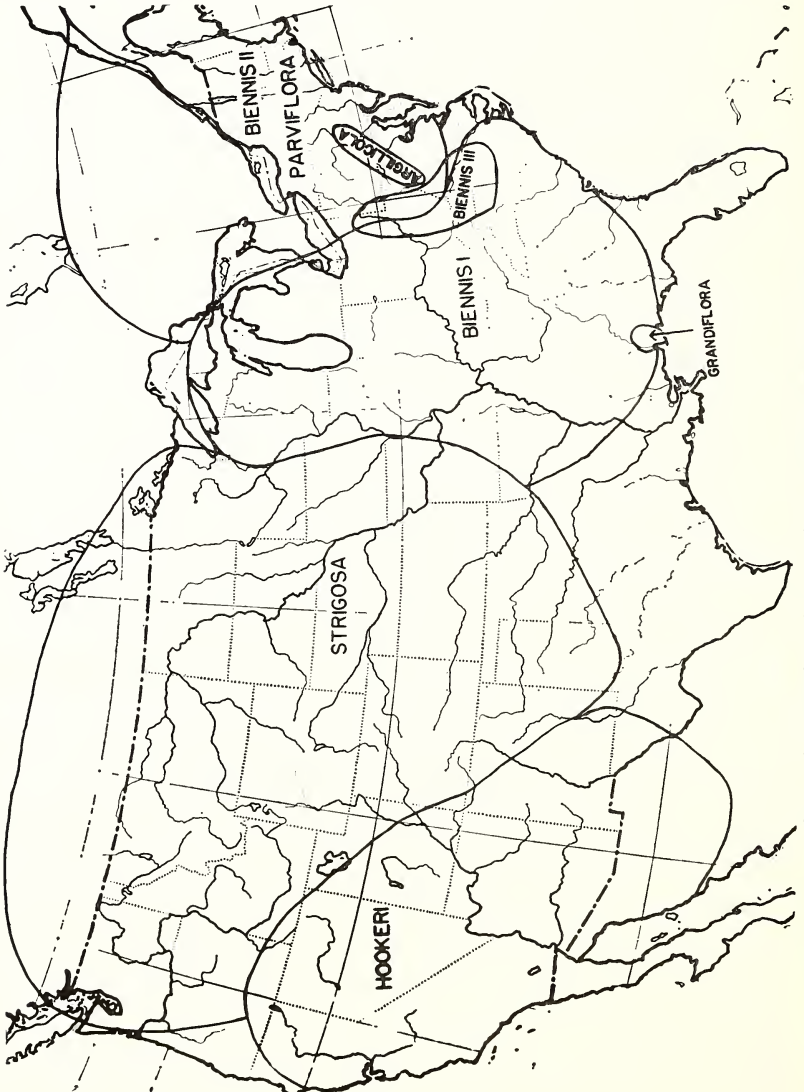


Fig. 7. Distribution of the major groupings of *Eucnothera*.

complexes in terms of the standard. Thus the standard complex (*hookeri*) was given the arrangement 1.2 3.4 5.6 7.8 9.10 11.12 13.14, where the ends of the chromosomes are numbered and arranged in sequence. One chromosome has ends 1 and 2, another 3 and 4, etc. Some complexes proved to be very similar to *hookeri*, e.g., the egg complex of *chicaginensis* has 1.2, 3.4 5.6 7.10 9.8 11.12 13.14. Other complexes differ completely from the standard, e.g., one of the complexes of the *grandiflora* of de Vries (*truncans*) has 1.13 3.7 5.2 4.6 9.14 11.10 8.12. Over 160 different arrangements have been found among the complexes analyzed. When these complexes are compared with one another from the standpoint of similarity or dissimilarity of end arrangement, geographical position and the external appearance of the plants of which they are a part, we find that these complexes and the races to which they belong fall into certain rather distinct groupings, each group of races having its own special geographical range, its own distinctive appearance and certain characteristic types of segmental arrangement in common. Ten such groupings have been found. They have been given tentative designations as follows (fig. 7): *biennis*-like *parviflora*, *strigosa*-like *parviflora*, *argillicola*, *biennis I*, *biennis II*, *biennis III*, *grandiflora*, *strigosa*, *hookeri*, *elata*. When we analyze the various segmental arrangements and try to see what interchanges have occurred to form them, we begin to see how these groupings have come about and I will now briefly tell the story of the way in which they have evolved.

The first *Oenothera* population that spread over the North American continent had certain rather distinctive structural features that showed it to be closely related to the subgenus *Raimannia*. We may call it the *proto parviflora* population, or population 1. Its leaves were narrow, relatively hairless, its stems tended to bend down behind the tips and then up at the tips, its sepal tips were subterminal—set off to one side of the tip of the bud. This population was apparently open pollinated with paired chromosomes for the most part. However, interchanges occurred here and there that introduced into the population a considerable variety of segmental arrangements. Large circles probably did not develop, nor did lethals become established. There is a relic of this population still extant in the shale barrens of the Appalachians. It is known as *argillicola*. It has large flowers, open pollination and small circles or none. Lethals are for the most part absent. It has bent stem tips and subterminal sepal tips.

At a later time, a second population developed which we may call the *proto-biennis* population, probably in what is thought of as the center of migration, namely, Central America. This probably arose during one of the periods when the Pacific Southwest was experiencing a wet climate, and this population was adapted to such conditions. As it spread into the area of the United States it came into the territory occupied by the first population. The second population, like the first, was open pollinated, free of lethals, and had small circles or none. A certain amount of heterogeneity in segmental arrangement probably developed, especially as the population spread eastward, although there seems not to have been too much in the way of segmental interchange in the middle western areas. Individuals of this second population crossed here and there with individuals of the first population, which they could do because both popula-

tions were open pollinated. Because, however, the interchanges which had taken place in the two populations were by chance entirely different, the segmental arrangements of some of the plants that crossed were different enough to give the progeny of these crosses a circle of 14, which is formed when none of the chromosomes contributed by the male parent coincide with any of those contributed by the female in segmental arrangement. In this way, circles of 14 arose suddenly and not by the gradual accumulation of translocations in a single line of descent.

Once circles of 14 appeared, the stage was set for the retention of lethals, as explained earlier. Some of these may have been in existence in population 2 before crossing took place, in the form of a special kind of sterility factor known as an S factor. A pollen grain that has a certain S factor cannot grow a tube down through the style of a plant possessing the same S factor. This makes it impossible for such a plant to reproduce by self pollination, and in a naturally open pollinated race this is an advantage because self pollination would lead to weakening resulting from loss of hybrid vigor. If S factors were present in population 2 when a cross occurred between it and population 1, the progeny would receive the S factor in the complex derived from population 2. This complex would not be capable of transmission through the pollen because of the S factor, and the hybrid would automatically have a pollen lethal. That population 2, the *proto-biennis* population, did possess S factors is suggested by recent work of Steiner.

The so-called egg lethal that developed in these hybrids and which was formed in the complex derived from population 1, may not have been in reality a lethal at all. It may have been merely a case where the complex derived from population 1 was unable to compete with the one from population 2 in the formation of eggs. An ovule or incipient seed in a flowering plant produces 4 cells, each of which is capable theoretically of developing the structure that contains the egg. In *Oenothera*, 2 of these 4 cells will receive one complex, 2 will receive the other. A competition then develops between the cells containing the two complexes. One of them will win out and produce the only egg developed in the ovule. It has been found experimentally that a given complex competes with different degrees of success with different complexes. If in the hybrids produced between populations 1 and 2 the complex from population 1 competed badly with the one from population 2, we would have what would seem to be an egg lethal in the population 1 complex, but in reality it would not be a lethal in the sense that it would actually destroy the egg. And so we see that a balanced lethal situation might have been produced at the very start of certain hybrids between populations 1 and 2. An S factor brought in with the complex from population 2, coupled with inability of the complex from population 1 to compete in egg formation with the complex from population 2, would give to a hybrid with a circle of 14 a so-called balanced lethal situation. With each such hybridization we would then have the beginning of a race typical in cytogenetic behavior of present day races except for the absence of the self pollinating habit. The latter came subsequently, and whenever it appeared, it gave such advantage to the plant in overcoming sterility due to the lethals that it was retained. In the struggle for existence, plants possessing self pollination coupled with a circle of

14 and balanced lethals survived, those without self pollination lost out.

It is not to be thought that only one cross took place between populations 1 and 2, or that the whole of a population crossed with the whole of another. Only here and there did individual plants cross. In some cases the hybrids thus produced had a circle of 14. From these are derived present day races. Other hybrids no doubt had smaller circles and because they could not breed true, and because the maximum of hybrid vigor would not be attained in their case, they lost out in the struggle for existence. The overlapping of populations meant, then, that many crosses occurred throughout the range, some of which died out, others with a circle of 14 survived. From each or many of these, have come the present day races of the group that we call the *biennis*-like *parvifloras*. They combine *biennis* and *parviflora* characteristics, with the latter having the larger influence on the external features of these races.

A relic of population 2 in its pure form still exists in the region of Mobile, Ala., and goes under the name of *grandiflora*. It is large flowered, open pollinated, is free of lethals and has only paired chromosomes.

This story of overlapping of diverse populations and the creation by hybridization of a new population took place twice again at later periods. There came a time when the moist period in the south-west ended. Arid conditions became established. As a result, evening primroses of populations 1 and 2 died out in most of the western half of the continent, although small islands of plants resembling population 2 have survived in isolated, well watered spots in the west. With aridity, there developed in the center of origin a third population, this time adapted to drier conditions. It resembled the present day *strigosas*, except that it had open pollination, mostly paired chromosomes and no lethals. This population invaded the western plains areas and the northern Rockies, and as it spread, interchanges took place between its chromosomes different from those that had occurred in populations 1 and 2. As population 3 (the *strigosa*-like population) advanced eastward, it invaded the territories of populations 1 and 2 and found plants of these populations that had not crossed with each other, that still had open pollination, small circles or pairs and an absence of lethals. Individuals of population 3 thereupon crossed with individuals of populations 1 and 2 and in particular cases circles of 14 resulted. Perhaps the same sort of story took place with respect to the origin of lethals and self pollination that had taken place in hybrids between populations 1 and 2. As a result, new populations arose with circles of 14, balanced lethals and self pollination. Crosses between populations 1 and 3 gave rise to what we now call the *strigosa*-like *parvifloras*. Crosses between populations 2 and 3 gave rise to what we call the *biennis* group. Population 1 gave to both the *biennis*-like and the *strigosa*-like *parvifloras* what we call the *parviflora* characters—narrow, hairless leaves, bent stem tips and subterminal sepal tips. Population 2 had what we call the *biennis* characters—broad, thin, crinkly leaves with little hairyness, and brittle stems. These characters tended to dominate in the races established by the crossing of populations 2 and 3, and which are known as *biennis* races. Population 3 had moderately narrow, thick and hairy leaves, and tough, woody stems. We call these the *strigosa* characters. The *biennis* group resulting from the crossing of populations 2 and 3

combined a *strigosa* with a *biennis* complex, the latter tending to dominate.

This story was repeated once again. A fourth population arose later, similar in external characters to population 3, i.e., a *strigosa*-like population, but with a history of interchange different from population 3. Population 4 overlapped population 3 and because plants were still in existence in both populations that were open pollinated, crosses occurred. Plants were formed here and there with circles of 14, and lethals and self pollination followed, as had been true in the earlier hybrid populations. The present day *strigosas* are the result. These have a circle of 14, balanced lethals and self pollination, but both of their complexes, so far as their effect on external appearance is concerned, are *strigosa*-like.

There is one population that has developed as did the earlier populations, with open pollination, absence of lethals, mostly paired chromosomes, though with a certain amount of heterogeneity in segmental arrangement, but which has never had an opportunity to overlap and cross with another population of open pollinated plants having a different history of interchange. This population has developed west of the continental divide and is characteristic of the Pacific south-west. It is the *hookeri* group. This group shows primitive characteristics, not only in having paired chromosomes, no lethals and large, open-pollinated flowers, but also in its segmental arrangements, the commonest arrangement being the one that on other grounds is considered to be the original one. It may be that this is an early population that, because it was on the west side of the divide, failed to come into contact with populations developing on the eastern side, or it may represent a relatively new population. In any event, the *hookeris* help us visualize what each of the 4 populations just mentioned was probably like at the time of its migration and overlapping with other populations.

There is another population which is found in Mexico and Central America to which Munz has given the name *elata*. This is of uncertain position. In appearance it shows similarities to *hookeri* and *strigosa*. So far as it has been studied, it has rather large, open pollinated flowers, paired chromosomes and no lethals. Its segmental arrangements, however, tend to depart from what is considered to be the original arrangement by two or three interchanges. Decision as to its status, its relation to other groups, must be withheld until more is known about it.

Two additional points that have to do with the evolution of the *biennis* group have not been mentioned. It has been found that *biennis* races fall into three rather distinct groups. *Biennis* I is characteristic of the middle west. Its *biennis* complexes have pollen lethals, perhaps S factors originally. Its *strigosa* complexes fail in most cases to compete successfully in egg production. *Biennis* II is characteristic of the northeastern part of the continent. It is scarcely distinguishable from *biennis* I in external appearance, but it is its *strigosa* complexes that have the pollen lethals and its *biennis* complexes that fail to compete in egg formation, just the reverse of the situation in *biennis* I. It may be that *biennis* II races are descended from ancestors in which the S factors which had come to function as pollen lethals became transferred from the *biennis*-like to the *strigosa*-like complex by the process of crossing over.

Segmentally the *biennis* I egg complexes are close to the original; the

biennis complexes of *biennis* II are farther removed from the original and show more variation in segmental arrangement from complex to complex. The *strigosa* complexes of both *biennis* I and *biennis* II are somewhat similar segmentally and rather similar to the complexes which are transmitted through the sperm in *strigosa*. For this reason the sperm complexes in *strigosa* are supposed to be derived from population 3 which overlapped the earlier *biennis* and *parviflora* populations; it was in turn overrun and contaminated by population 4 which is now represented by the egg complexes of the *strigosas*.

Finally, there is, in the areas where *biennis* I and II overlap, a third class of *biennis* races, designated as *biennis* III. These, like all other *biennis* races, have a circle of 14, balanced lethals and self pollination. They do not, however, possess a *strigosa*-like complex: both of their complexes are *biennis* complexes, so that *biennis* III races are pure *biennis*. *Biennis* III has no doubt arisen in comparatively recent times from crosses between *biennis* I and *biennis* II. Their egg complexes are very similar in segmental arrangement to the egg complexes of *biennis* I, and their pollen complexes are very similar or often identical in segmental arrangement with the pollen complexes of *biennis* II. *Biennis* III has come about, therefore, by crosses between *biennis* I as female and *biennis* II as male, a fact attested by its geographical location in the regions where *biennis* I and II overlap. Theoretically one should also get in this region the reciprocal cross between *biennis* II as female and *biennis* I as male. Actually we have found only one such plant and it had a circle of 6 and 4 pairs. The *strigosa* complexes in *biennis* I and II are similar enough segmentally that hybrids bringing them together will have small circles; and because plants with small circles do not breed true and do not have full hybrid vigor, they apparently do not survive readily in nature. Consequently this particular theoretically possible population has not caught on.

But it may be asked how self-pollinating races such as *biennis* I and II can cross. This leads me to the final point in the story, namely, that although the races formed by the crossing of ancestral populations are self pollinating, they are nevertheless capable of crossing with other races if the barrier set up by self pollination is broken down. Actually, the barrier does break down occasionally. While in general two races will not cross, even if growing together, a cross will occasionally occur when for some reason or another a flower fails to develop enough pollen to take care of all its eggs. Then, when the flower opens and insects visit it, the pollen they bring with them may have a chance to function. Hybrids are thus produced in nature, but only rarely. If these hybrids have a circle of 14, they may become the beginning of new races, and successful crosses between *biennis* I and II have in this way been occasionally produced. In many cases, however, outcrosses will produce hybrids with other configurations than a circle of 14, and these are usually unable to survive.

In the North American *eurotheras*, then, the story of evolution is becoming clear in its main outlines. Successive populations, each overlapping earlier ones, and being overlapped by later ones, have crossed, forming hybrids which have given rise to races that have survived to the present day. The *Oenothera* population as a whole consists of true-breeding, isolated lines, rarely crossing with each other. Their present

condition is the result of the combination of the following factors: (a) chromosomes that were originally of uniform size and shape, with central centromeres and a tendency to break more easily in the centromere region than elsewhere; (b) translocations which were able to survive and accumulate because they did not disturb the morphology of the chromosomes; (c) hybridization between populations that had suffered different histories of interchange, thus producing hybrids with a circle of 14; (d) retention of lethals when circles of 14 were present because they ensured maximum hybrid vigor and true breeding; (e) self pollination which was unable to reduce hybrid vigor when balanced lethals were present, but which encouraged rich pollination, and with the lethals made the races true-breeding. Most of these characters by themselves would be harmful to the plant, but when brought together, and when originating in the particular sequence that I have outlined, they have made for a high degree of vigor and survival value.

I would only point out one thing more. *Oenothera* has to a certain extent sacrificed racial well being for individual well being. The development I have outlined has made for vigorous individuals well able to compete in the struggle for existence. The penalty that has been paid is that the populations that have developed are composed of innumerable isolated lines. When a mutation occurs it is usually recessive and will show out only when an individual is formed pure or homozygous for this change. This ordinarily cannot take place in *Oenothera*, for pure races and purity of genes are very difficult to achieve in view of the enforced heterozygosity. Only when a rare outcross occurs is there a chance of chromosome pairs being formed and recessive genes being able to show up. But hybrids that have pairs usually are unable to survive. The best, and almost the only chance that a recessive gene will produce an effect will be if it gets into combination, through outcrossing, with a different gene in a new hybrid with a circle of 14. It may possibly be able to exercise some influence in a new combination.

The future evolution of *Oenothera* will probably take the form of a gradual increase in the number of true-breeding isolated lines, through rare crosses producing hybrids with a circle of 14. If conditions should change radically, the relative inability of recessive genes to show out might reduce markedly the plasticity of the genus and its ability to adapt to change. The main hope of the subgenus would be that among the plethora of races then existent there might be some that would be able to survive the changes, and hence preserve the group from extinction. It is not too much to say, however, that the inability of genes to circulate freely in the population and to show up occasionally when recessive would constitute a serious threat to the permanence of the genus should conditions change. Future survival of the race may have been sacrificed for increase of survival value of the individual.

I have briefly outlined a single story of evolution. It is in many respects a unique one and different from what has happened in other groups. It is important, however, to look for cases where one can analyze in detail the story of evolution. In this way we can learn more about the nature of evolutionary change and the factors that determine the survival or disappearance of the changes produced.