The one with the highest total frequency is ^{h}johansen, 1061 or 38.1% of the total, far in excess of that expected for randomness. Hence, the ^{h}johansen set was the original 7-chromosome ancestor in the evolution of the Oenothera races. The exchange between 1.2 and 3.4 to form 1.4 and 3.2 occurred very early in the evolution of certain groups. Of the other combinations of ends, these two (1.2 and 3.2) have the highest frequencies.

From their survey of the frequencies, they concluded that any end can become associated with any other end, and that there is no evidence that one segmental association can arise more easily than others.

Chiasma frequencies

Harte (1953, 1954, 1954a) found that the frequency of terminal chiasmata was less within the ⊙12 than in the bivalent, based on two hybrids. The same was found in hybrids with a ⊙4, but different complex-combinations with the same chromosome formula gave different frequencies. Also, structural homozygotes and bivalent chromosomes have a higher frequency of non-terminal chiasmata than do the multipartite configurations (Hoffmann 1954). Also, these chiasmata are more frequent in some complex-combinations than in others.

Linkage studies in *Oenothera*

The linkage studies reported by Renner (1925, 1928), Emerson (1930, 1931, b), Shull (1923, 1927, 1928), Sturtevant (1931a) and others have been reviewed by Emerson and Sturtevant (1932). Additional data were reported by Oehlkers (1933) and Renner (1958a).

A list of the genes which have been used in linkage tests is given in Table 47. The associations indicated are based on those races in which that chromosome was present as a separate pair, and the marker inherited independently of those in the ring. This is the result expected if each of the genes is closely linked to an interchange point in the ring. Ordinarily the number of linkage groups corresponds to the number of separate rings and pairs which are genetically marked. The two exceptions are a factor for small flower size (Co) and the factor for brevistylis (br) which are linked with each other with about 15% recombination, but have shown no linkage with any of the other known factors even in plants with a ring of 14 chromosomes. There have been two cases where the F_1 showed one more linkage group than there were separate chromosome groups, but both involved either Co or br. Apparently Co and br are too far from the interchange point to show linkage. Catcheside (1954) has located Co in the .12 chromosome end by using trisomics derived from O. blandina.

With the exception noted above, most of the linked genes show very little crossing over even when they are in paired chromosomes. In the 1.2 chromosome, Shull (1927) found very little crossing over between old gold (v), bullata (bu) and double flowers (sp). Emerson and Sturtevant (1932) reported very little between R and v. In the 3.4 chromosome, Emerson (1932a) found linkage between s and n, 8% between P and s. When this chromosome was in a ring of 10, there was about 5% recombination between p and s. Whenever plants carrying the genetic crossover were checked cytologically, they had the same configurations as their non-crossover sibs. Hence, the crossovers had not occurred in a differential segment. Also, the genes must have been located in the end or interchanged segments.

Emerson (1931b) also reported the results of a 3-point linkage test in plants with sulfurens. franciscana (⊙10) in which each marker was probably located on a different

Table 47. Genes used in linkage tests in Oenothera, togther with the chromosomes carrying them, where known.

Gene symbol	Character	Chromosome	End
R	red midribs	1.2	.2
v	old-gold flower color	1.2	
bu	bullata	1.2	
sp	double flowers	1.2	
a dominant gene	suppressing Co.	2.3	
n	nanella dwarf	3.4	.4
P^r	punctate stems, red buds*	3.4	.3
P^s	punctate stems, striped buds		
P	punctate stems, green buds		
p	not punctate stems, green buds		
f	revolute	3.4	.4
Ss	yellow vs. sulfur flower color	3.4	.3
Sp	pointed buds and leaves vs. blunt	5.6	
Cr	cruciate petals	5.6	
br	brevistylis		.12 .11
Co	small flower size		.12 .11
d	dwarf stature	13.14	
B	broad leaves		
Cu	curved stems		
M	marginate leaves		
	velans zygotic lethal		

arm in the ring. For splashed budcones (Spl), green bud cones (Gr) and sulfur-flowers (s) there were 3.02% singles in region 1, 4.00% in region 2 and 0.49% doubles. The expected number of doubles is 0.16% with no interference. The data indicate not only no interference across the points of breakage, but an excess of such crossovers, so-called "negative interference". Emerson also reported the results of a linkage study in a ⊙4 involving chromosomes 2 and 3 in Drosophila set up in a similar manner. The interchange heterozygote carried three gene markers, one on each of three of the four arms. In a total of 2051 backcross flies there were 6.39% singles in region 1, 1.86% singles in region 2, and 0.40% doubles where 0.13% doubles were expected with no interference. Here again there was an excess of doubles as observed in Oenothera.

As a consequence of the cytological constitution, many characters are inherited in groups in Oenothera. The larger the ring the larger the number of characters that may be inherited as a group. This results from the fact that in interchange heterozygotes there are only two viable combinations of chromosomes from the ring, namely, the parental combinations. Also the number of linkage groups demonstrable for a given race usually depends on the number of separate rings and pairs. A list of the characters associated with each of the complexes in two races is shown in Table 48.

The characters listed in Table 48 for each complex are inherited as a unit since

Table 48. List of characters produced by the complexes of certain races of Oenothera.

Complexes:	lamarckiana (⊙12 + II) velans	lamarckiana (⊙12 + II) gaudens	Iowa 1 (⊙14) α Iowa 1	Iowa 1 (⊙14) β Iowa 1
rosette, 1st. yr.	none	often		
Central shoot				
habit			straggling	
stem structure			shorter	taller
tip color			green	green
basal color			red	green
papillae			red punct.	red punct.
hairiness			not felty pubescence	felty pub.
Leaves, width	narrow	broad	broad	narrow
color			d. green	gray-green
habit			crinkly	wavy
midribs	white	white or red	red	white
punctate margins	punctate	not punct.		not red punct.
Bracts				
size			small	larger
tips			red	
Spikes			narrow	broader
bud-color	red	green	green	green
shape			stout	longer
sepal tips			spreading	appressed
styles			short	short
flower size			small	small
fruit shape			stout	narrower
zygotic lethal	yes	yes		
transmission	♀*	♂*	♀	♂

* after 1934

the races in which they occur have all or all but one of the chromosomes in a ring at meiosis. How they are transmitted depends on which complex can function through the male and which through the female. The complexes differ in the genes they carry for quantitatively inherited characters as well as qualitative ones. Emerson (1929a) reported that linkage extended to these quantitative characters as well. Segregation in lines which had a large ring was limited to a few main types, frequently only two, whereas the same characters in a race with 7 II or a ⊙4, showed the wide range of segregation typical of quantitative inheritance.

Taxonomy of *Oenothera*

Since the various races and species with rings are heterozygotes, the taxonomic relationships cannot be determined from their phenotype alone, but must depend on an analysis based on the progeny of crosses planned to determine the genes carried by each complex.

Based on the cytogenetic studies, and a taxonomic study of the various races and their hybrids, Cleland (1935, 1940), Cleland, Preer, et al. (1950) concluded that, in the genus Oenothera, subgenus Euoenothera (formerly called Onagra) the North American

Table 49. Phenotypic characteristics of various groups of North American wild races of Oenothera.

Characters	hookeri	small irrigua	strigosa	biennis group 1	biennis group 2	biennis group 3	parviflora	grandiflora
Locality	Western	New Mexico	Rocky Mt. Plains States	Midwest	Eastern and N.E.	Eastern	Northeastern	Southeastern
Flowers	large	large	small or intermediate	small	small	small	small	medium
Styles			short					
Leaves			narrow, smooth	broad, thin	broad, thin	broad, thin	narrow	narrow
Pollination	open	open	self	self	self	self	self	self
Lethal	absent	usually absent	balanced	balanced	balanced	balanced	balanced	balanced
Breed true	usually, but homo.	no	yes	yes	yes	yes	yes	yes
Configuration	pairs or small rings	intermediate ⊙ 6 mainly, some ⊙ 4, ⊙ 8, ⊙ 10	⊙ 14	⊙ 14	⊙ 14	⊙ 14	⊙ 14	⊙ 14

wild races fall into 8 groups tentatively designated as: hookeri, a small irrigua group, strigosa, biennis group 1, biennis group 2, biennis group 3, parviflora, and grandiflora. Several of the characteristics of the races in each of these groups are summarized in Table 49.

The taxonomic situation in the forms with large rings, was summarized by Cleland (1944) as follows:

1. There are many true-breeding races, more or less isolated from each other by their self-pollinating habit. Some natural crossing occurs.
2. Each race contains two complexes differing from each other in segmental arrangement, and also in genetic makeup.
3. New forms may arise by occasional natural crossing, gene mutation, crossing over, or new interchanges.
4. Since these heterozygotes breed true, one complex may be masked by the other. Two races that look alike may differ in the complexes they carry.

The forms with large rings have balanced lethals and are self-pollinated while those with the small rings or with all pairs are open-pollinated. The large ring maintains a maximum of heterozygosity with a minimum of sterility. Shull (1923) stated that the lethal-carrying forms were usually superior in vegetative vigor to the a-lethal ones. The heterozygous genotype is perpetuated and hence the survival of combinations showing heterosis might be expected. The presence of a balanced zygotic lethal in each of 7 independent pairs of chromosomes would reduce fertility to less than 1%. In a ⊙14, zigzag orientation should restore normal fertility. True-breeding behavior might be brought about by the presence of a zygotic lethal in each complex, but then only 50% of the embryos would be viable. In Oenothera, normal seed setting has been brought about by gametophytic lethals, one in the microspores and one in the megaspores. The one segregating in the megaspores produces normal seed set only if combined with strong megaspore compeition favoring the megaspore genotype without the lethal.

Large rings in other genera of the *Onagraceae*

Considerable work has been done on the South American Euoenotheras. They do not cross readily with the North American ones. Of the races studied, nine had a ⊙14, three a ⊙12, one a ⊙10 + ⊙4, one a ⊙10, and five had 7 II (Hagen, 1950). Those with a big ring were true-breeding, complex-heterozygotes. Analyses of other subgenera of the genus Oenothera, some in South America and some in North America, showed that some had a big ring, others not. Rings have been found in other genera in the Onagraceae, in Gaura, and in Godetia. In Gaura the largest rings reported were a ⊙12 in one and a ⊙14 in another, along with alternate segregation of the chromosomes in the ring and true-breeding behavior (Bhaduri 1941, 1942). Godetia whitneyi is found along the Pacific coast of the U.S.A. from San Francisco to Vancouver. Races with 7 II, ⊙4, ⊙6, ⊙6 + ⊙4, ⊙8, ⊙10, or a ⊙12 have been found. In all of these, the chromosomes at meiosis are described as usually showing zigzag orientation (Håkansson 1931, 1942, 1947; Hiorth 1947, 1948). One complex was found to be common to these strains. This is in contrast to Oenothera where both complexes vary from race to race. Certain of the Godetia collections had lethals, but had not developed a completely balanced system. This is regarded as a species in which the Oenothera type of behavior is developing, or gradually becoming established.

Godetia furnishes one example of a viable strain lacking one chromosome pair (nullisomic). Supposedly, the essential material of that chromosome had been transferred to other chromosomes (Hiorth 1948, Hakansson 1946).

Large rings in species in other families

Large rings have been reported in other families also, for example, a ⊙10 in Chelidonium majus (2n=14) by Nagao and Saki, (1939); a ⊙12 in Rhoeo discolor (2n=12) a monotypic genus, by Darlington (1929 a, b), Sax (1931) and Simmonds (1945); a ⊙16 in Hypericum punctatum (2n=16) by Hoar (1931), and a ring of 10 in Paeonia californica (2n=10), a long lived perennial, by Stebbins and Ellerton (1939) and Walters (1942).

In Campanula persicifolia ---- (2n=16), Darlington and Gairdner (1937) and Darlington and LaCour (1950) produced larger rings by successive intercrosses between races which had different rings of four and rings of six. They finally obtained plants with a ⊙12. From self pollination of the large rings or of the wild races with a ⊙4 no normal plants with 8 II were found. Since the species is self incompatible they concluded that under the forced crossing, deleterious genes had accumulated in regions near the translocation breaks. In the ⊙4, zigzag orientation was in excess over adjacent segregation when the configuration was a ring but not when it was a chain. They also emphasized the point that in this species with a high seed potential of 2,000 to 20,000 per plant, a high frequency of deleterious or lethal genes should not be a serious bar to its survival.

Darlington and Gairdner (1937) also reported two cases in Campanula in which a ⊙8 broke down into two interdependent rings of four, that is the new races had two separate rings, yet formed only two functional gametic complexes. This might be accomplished by a genetic mechanism of complementary lethals, but would require a complex hypothesis. They suggested that it could arise by crossing over between two non-homologous chromosomes which carried a duplication that had arisen by "redundant translocation", a process which they did not explain. Such a duplication may arise from an F_1 between interchanges involving the same two chromosomes but with breaks at different positions, as described by Gopinath and Burnham (1956). The duplication might become established as a homozygote or be carried along as a heterozygote. If two of the chromosomes in the original ⊙8 in Campanula had such a duplication, crossovers between the chromosomes carrying the duplication would break the ring down into smaller configurations. Certain combinations of these crossover gametes with a non-crossover gamete from the ⊙8 would produce the interdependent rings of four.

If one of the breaks in the second interchange which produced the original duplication had not occurred at the same point as the first one, other regions could have been duplicated, increasing the number of different kinds of gametes which might be produced by crossing over. Emerson (1936) reported that O. biennis (albicans. rubens) with a ⊙6, ⊙8 forms only two kinds of gametes, whereas four would be expected. The explanation outlined above may explain this also.

In maize, a ring of 8 chromosomes has been produced by irradiating a line homozygous for one interchange. Plants with a ⊙8 when selfed or crossed with normals produce about equal numbers of normal plants and plants with a ⊙8, and occasional ones with a ⊙4 (two types) or with a ⊙6 (also two types) (Lazaro 1944, Marino 1947, and Mohamed 1952). Also see page 113 for a discussion of large rings in maize.

Explanations for the *Oenothera*-type behavior

The behavior of the big rings in Oenothera raises two interesting questions:

1. Why is chromosome segregation from the ring mostly alternate?
2. How did the system of true-breeding complex heterozygotes become established?

Those species which show predominantly alternate segregation of the chromosomes at meiosis appear to have certain cytological characteristics in common. The chromosomes are relatively uniform in length, the centromeres are median or nearly so, and the chiasmata are localized at the ends, i.e. non-terminal chiasmata are rare. Also the interchanges that have survived in Oenothera are those with breaks near the centromeres, and consequently the exchanged pieces were long and nearly equal in length. In those with breaks farther from the centromere, crossing over in the proximal segments is probably relatively rare.

It is possible that the orientation is a matter of timing. In some species, all may be synchronized to pass on the plate together. In others, it may be a progressive process, and the first one then determines that the successive ones will be oriented in an alternate manner. It is also possible that in certain species directed segregation is determined genetically. This cannot be true in Collinsia heterophylla, since the rings produced by X-rays show an excess of alternate segregation (Garber and Dhillon 1962), whereas those produced by colchicine show about 50% of alternate segregation (Soriano 1957).

In barley, a species reported to have about 75% of alternate segregation in rings of four (Smith 1941), the centromeres show little evidence of active stretching toward the poles, whereas in corn, a species with about 50% of alternate segregation, centromere activity is very strong (O. Miller, unpublished). This suggests a difference in balance between centromere activity and the repulsion forces acting on the chromosome. The behavior of the chromosomes in a ring may depend on the balance that has become established between these two forces in a particular species.

In some species, including Oenothera, there is a high frequency of interlocked bivalents which may result in chromosomal interchange. The presence of duplicated segments, of natural radiation, and the occurrence of sudden temperature shocks may also contribute occasional interchanges. Heterosis probably plays an important role in the survival of interchanges. Whenever there is greater vigor in an interchange heterozygote the heterozygotes tend to survive. Chromosomes tend to be added to the first ring if they add additional heterosis. The interchange heterozygotes would then be better competitors than individuals without the interchanges. Self incompatibility in the original plants would bring about the outcrossing which could bring together new gene combinations.

Also see:
Cleland, R. E. 1972. Oenothera Cytogenetics and Evolution. 370 p. Academic Press, N.Y.