Chromosome Linkage in Certain Oenothera Hybrids

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VII. *Chromosome Linkage in Certain Oenothera Hybrids.*

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(Plates 89 and 90.)

Part I.—Genetical.

Introduction.

The genetical peculiarities of the genus Oenothera have long been recognized, but further work will, no doubt, reveal similar conditions in other groups. The immense amount of genetical work done with the Oenotheras by de Vries (1913) and many others has given a body of data unequalled in any other group of plants. Every phase of the life history has been intensively investigated (see Gates, 1928) and the results of great numbers of species- and variety-crosses are known. Two of the most striking peculiarities discovered by de Vries (1907) and confirmed by others (Gates, 1913) were (1) the occurrence of twin types in the F_1 of crosses between different species, and (2) the fact that many of the F_1 forms produced by crossing breed true or segregate in only one or two characters.

By crossing, numbers of Oenothera species have been analyzed, particularly by Renner (1925), into two " complexes," both of which may be active in different egg cells of the plant, but frequently only one of them is functional in the pollen. The basis of this failure of Oenothera hybrids to segregate in Mendelian fashion has long been obscure, but it is now becoming clear that it has a cytological basis in the linkage of the chromosomes during meiosis, which is a common feature of Oenothera species, mutations, and hybrids. It is only necessary here to mention that each species has its characteristic linkage arrangements, and that fresh linkages different from those of the parents occur in the mutations of *Oe. Lamarckiana* and also in hybrids between different species. It is to be anticipated that the more numerous such chromosome linkages are, the smaller will be the amount of ordinary Mendelian free assortment of factors.

The object of the present paper is to describe reciprocal Oenothera hybrids which are very unlike, both of which breed true except in certain minor features, and which show different chromosome linkages. This is the first case in which unlike linkages have been

recorded in reciprocal hybrids.   Since a large amount of chromosome linkage is found in many Oenotheras, it appears probable that some wild Oenothera species have arisen through natural crossing, and form true-breeding species because of the linkages which occur between their non-homologous chromosomes.   Although these conditions are at present unique in Oenothera, probably some other genera will be found to show similar phenomena when intensively investigated.   The chromosome number in all the hybrids with which we are here concerned is $2n = 14$.

The hybrids described are *Oe. ammophila* ♀ $\times$ (*biennis* $\times$ *rubricalyx*) ♂ and its reciprocal. The crosses were made in 1922 and the F_1 grown in 1923, at the Royal Botanic Gardens, Regent's Park, where all these experiments have been carried out.   The (*biennis* $\times$ *rubricalyx*) used in these reciprocal crosses was the F_5 of a true-breeding strain.   Reciprocal crosses between *Oe. biennis* and *Oe. rubricalyx* had been made in 1912 and used for a study of the inheritance of flower size.   The results of these investigations of inheritance in length of petal were published (GATES, 1917, 1923), and the pedigrees and genetic behaviour of the families are there briefly outlined.   The size inheritance studies were based on measurements of the flowers in four successive generations of *Oe. biennis* $\times$ *rubricalyx* and the reciprocal.   The eight F_4 cultures, numbering 815 plants, were very uniform in every feature except bud colour and flower size.   The bud colour shows sharp segregation of the *rubricalyx* type of red pigmentation R (described in earlier papers) from the green-budded type (r).   Two families (76 + 130 plants) bred true for R, another family of 121 dwarfs from a dwarf mutation were all R, two families (88 + 145 plants) bred true for r;   and three families showed segregation in bud-colour, the ratios being 133R : 6r, 64R : 2r and 2 doubtful, and 18R : 1r in the first row of a culture of 48 plants the remainder of which escaped classification.   These three ratios are all consistent with 15 : 1.   Similar ratios for R : r have often been obtained before, and a duplicate factor explanation was suggested (GATES, 1915a).

The Parents.

A new series of studies of flower-size inheritance was begun in 1922, by crossing this family of *Oe.* (*biennis* $\times$ *rubricalyx*) F_5 reciprocally with *Oe. ammophila* Focke.   From the previous paragraphs it will be clear that this race was as constant as an ordinary species in every feature except bud-colour, in which segregation occurred, and flower-size, in which there was a small amount of variation, though not more than would be regarded as ordinary fluctuation in any species which was not biometrically investigated.   Five generations have since been grown from this second series of hybrids, and the statistical results from an enormous mass of measurements of the petal-lengths from these and several other hybrids will be published on another occasion.

This paper is concerned only with the chromosome linkages and general genetic behaviour of one pair of these reciprocal hybrids and their descendants, namely, *Oe. ammophila* $\times$ (*biennis* $\times$ *rubricalyx*) and *Oe.* (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila*

through five generations. Fig. 1 is a conspectus of the families of plants descended from *Oe.* (*biennis* × *rubricalyx*) × *ammophila*, and fig. 2 (folding diagram) of the cultures descended from the reciprocal. They were grown in numbers because of the intensive study of flower-size inheritance which was being made, but they showed remarkably little variation in other features, except that some families split into twin types.

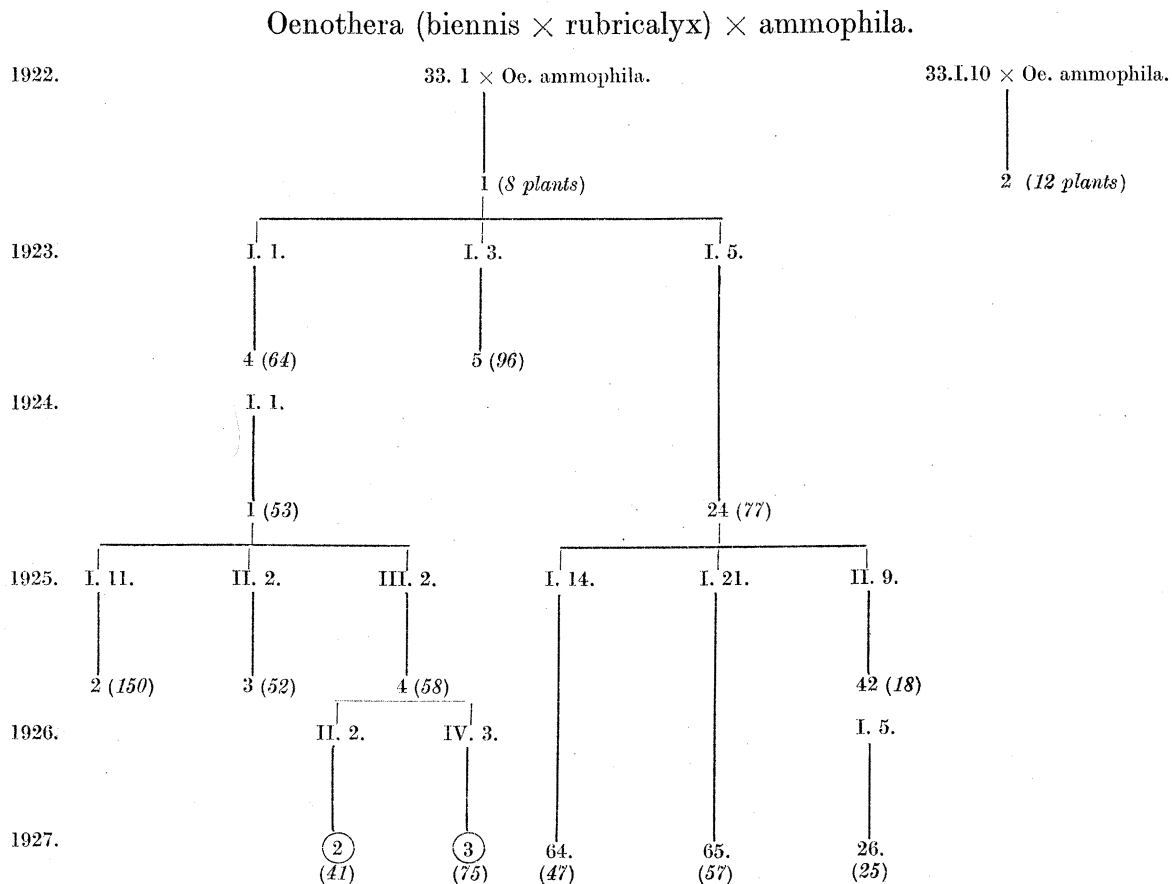Oenothera (biennis × rubricalyx) × ammophila.

Fig. 1.—Chart of families, showing in each case the number of the parent plant, the number and year of the culture, and the number of plants in the culture. (Compare Table I, p. 370.) Cultures 2·27 and 3·27 were examined cytologically.

The *Oe. ammophila* parent was from a strain of unknown origin grown in Regent's Park. It had the bent stem-tips characteristic of this species and was afterwards found to agree in all essentials with a strain received from Prof. O. Renner, of Jena, which he had grown from seeds collected on Heligoland in 1922.

Other features of *Oe. ammophila* are the narrow, lanceolate to elliptical grey-green leaves and the small flowers (mean petal-length of flowers on the main stem about 17 mm.). The rosette leaves are long-lanceolate, sparsely denticulate, about 20–22 cm. long, 30–35 mm. wide, soft pubescent when young, glabrous when mature. The leaves are devoid of red, the midribs being greenish white and the petioles narrowly margined.

The stems bear red papillæ, but are otherwise green.   The stem leaves are numerous (internodes short), elliptical to lanceolate and drooping, upper bracts sessile, otherwise like the upper leaves.   The flowers form a compact inflorescence at the bent tips of the stem and branches.

The parent strain of *Oe.* (*biennis* $\times$ *rubricalyx*) nearly resembled *rubricalyx* in habit and foliage, agreed with *rubricalyx* in the red sepals and diffuse red on the upper part of the stem, and differed from it in having somewhat smaller flowers.   The leaves differed from those of *rubricalyx* only in being slightly less crinkled.   This parent may be regarded as a slightly modified *rubricalyx* with considerably smaller flowers.

The F_5 culture (33·22) of *Oe. biennis* $\times$ *rubricalyx*, which contained the parent of the present experiments, numbered 14 plants.   Of these, 13 came into flower and their buds were 12R : 1r.   The petal-measurements on seven of these plants numbered 156 and gave a mode on 30 mm. length of petal, with a range of variation from 25–33 mm.

The F_1 generation.

Two F_1 families of *Oe.* (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila* (1·23 and 2·23) were grown in 1923 ;   the female parents were two different individuals in the same culture, while the pollen was taken from the same *ammophila* plant in both cases.   The F_1 cultures both contained twin types, which differed only in having light or dark green leaves, as shown in Table I.

Table I.

Culture.	Light Green.	Dark Green.	Total.
1·23	7	1	8
2·23	4	8	12
	11	9	20

The midribs were red both dorsally and ventrally.   Ventral red midribs are a character of *rubricalyx*.   A striking feature of these hybrids is that the bent stem-tips of *Oe. ammophila* are inherited through the pollen.   Fig. 3 shows a plant coming into flower. The stem comes out from the rosette at an angle of about 30°—45° with the horizontal, giving the plants a remarkable appearance.   As shown in the figure, the lateral branches are also bent at the tips.   A row of these plants gives a curious appearance, because in each one the oblique stem forms a different angle with the row, but its position is fixed and there is no nutating movement.
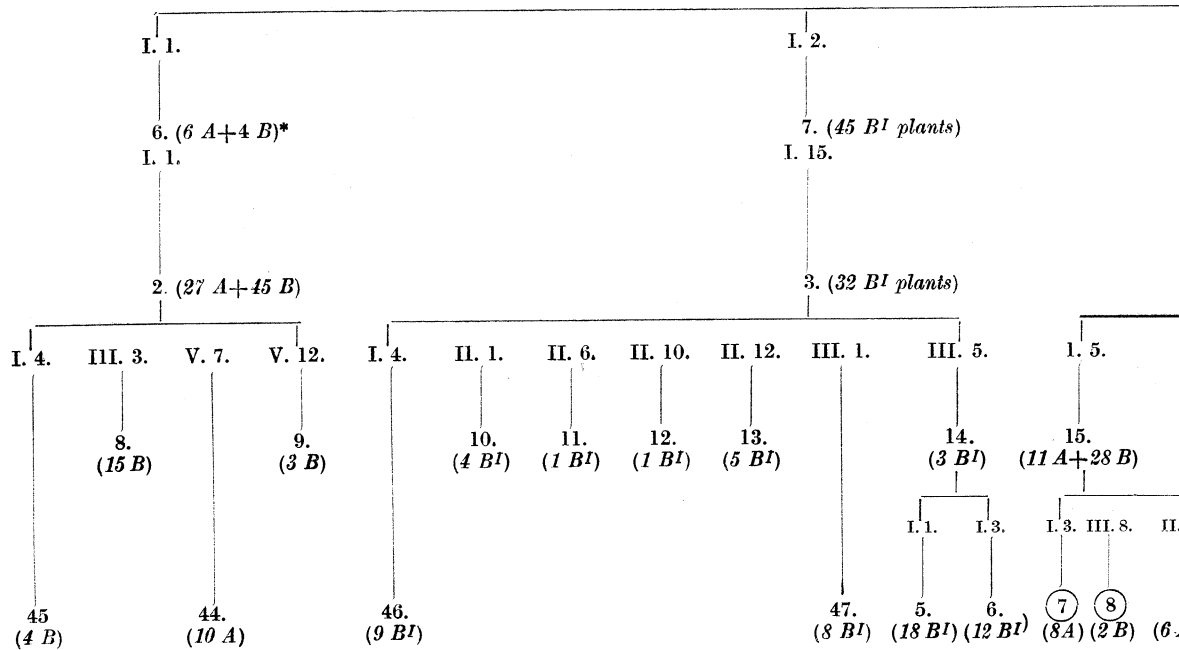
1922.

1923.

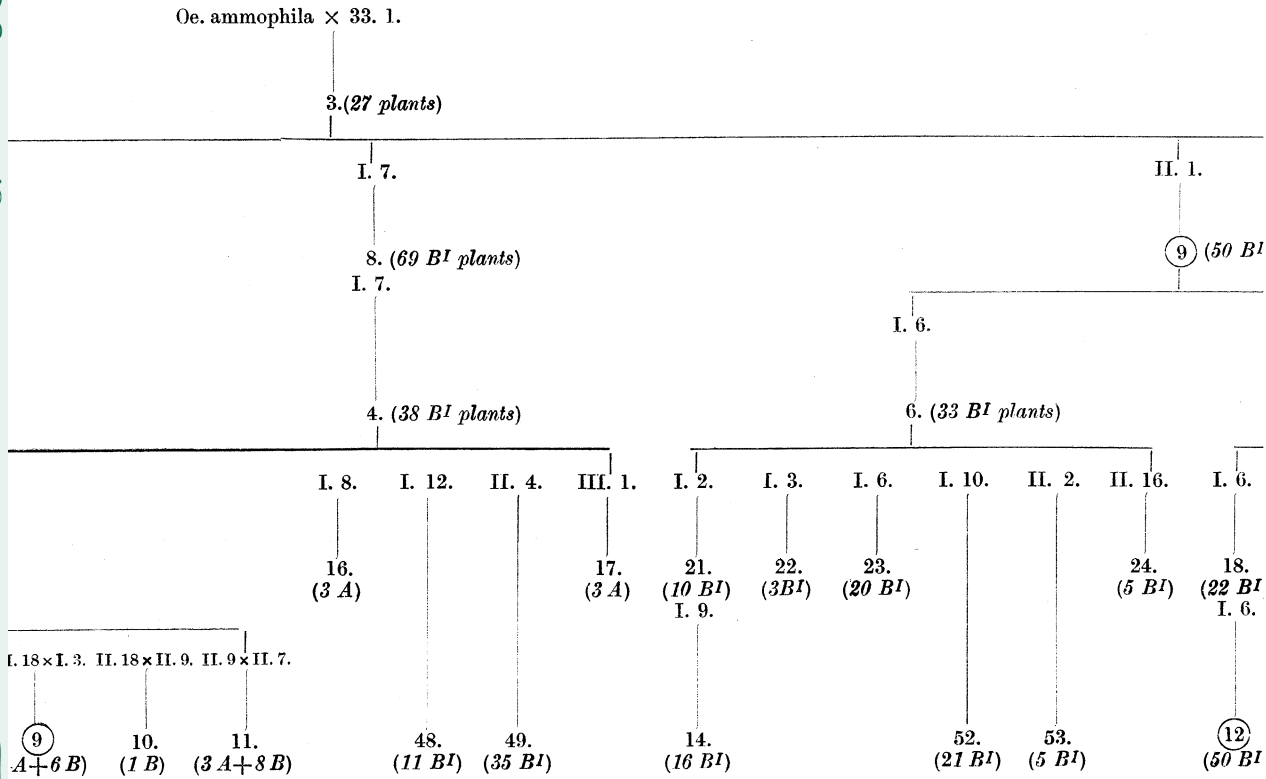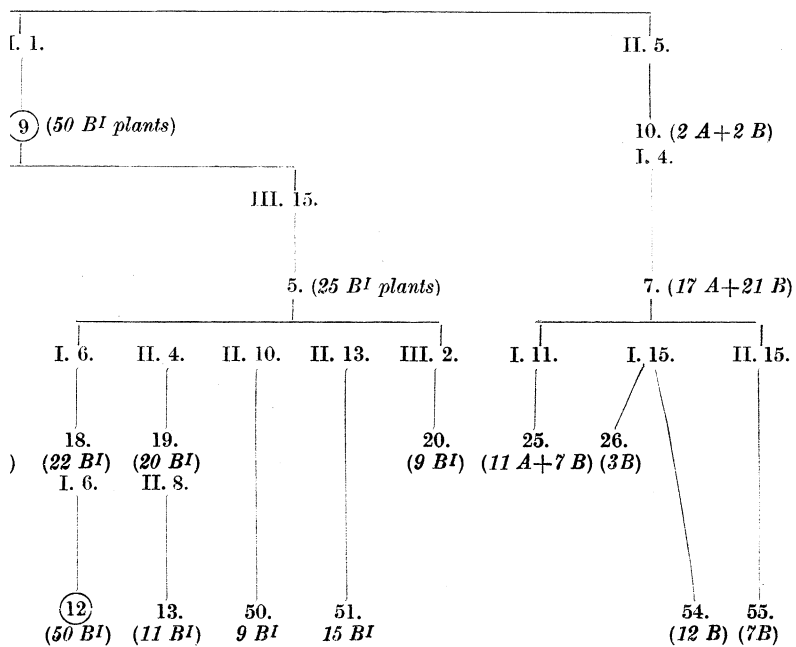I. 1.   I. 2.

1924.   6. $(6 A + 4 B)$*   7. $(45 B^I plants)$
             I. 1.   I. 15.

1925.   2. $(27 A + 45 B)$ 3. $(32 B^I plants)$

     I. 4.   III. 3.   V. 7.   V. 12.   I. 4.   II. 1.   II. 6.   II. 10.   II. 12.   III. 1.   III. 5.   1. 5.

1926.   8.   9.   10.   11.   12.   13.   14.   15.
             $(15 B)$ $(3 B)$ $(4 B^I)$ $(1 B^I)$ $(1 B^I)$ $(5 B^I)$ $(3 B^I)$ $(11 A + 28 B)$

                                                                                                  I. 1.   I. 3.   I. 3. III. 8.   II.

1927.   45   44.   46.   47.   5.   6.   (7)   (8) $(6$
        $(4 B)$ $(10 A)$ $(9 B^I)$ $(8 B^I)$ $(18 B^I)$ $(12 B^I)$ $(8A)$ $(2 B)$

                                                                                                                          * $

Oenothera ammophila × (biennis × rubricalyx).

Oe. ammophila × 33. 1.

3.(*27 plants*)

I. 7.

II. 1.

8. (*69 B^I plants*)
I. 7.

⑨ (*50 B^I*

I. 6.

4. (*38 B^I plants*)

6. (*33 B^I plants*)

I. 8.   I. 12.   II. 4.   III. 1.   I. 2.   I. 3.   I. 6.   I. 10.   II. 2.   II. 16.   I. 6.

16.   17.   21.   22.   23.   24.   18.
(*3 A*)   (*3 A*)   (*10 B^I*)   (*3B^I*)   (*20 B^I*)   (*5 B^I*)   (*22 B^I*
I. 9.   I. 6.

I.18×I. 3.   II. 18×II. 9.   II. 9×II. 7.

⑨   10.   11.   48.   49.   14.   52.   53.   ⑫
A+6 B)   (*1 B*)   (*3 A+8 B*)   (*11 B^I*)   (*35 B^I*)   (*16 B^I*)   (*21 B^I*)   (*5 B^I*)   (*50 B^I*

Three other plants were forming stems and could not be classified.

Fig. 2.   Chart of Families.

(The cultures in circles were studied cytologically.)

I. 1.   II. 5.

⑨ (50 B^I plants)   10. (2 A+2 B)
I. 4.

III. 15.

5. (25 B^I plants)   7. (17 A+21 B)

I. 6.   II. 4.   II. 10.   II. 13.   III. 2.   I. 11.   I. 15.   II. 15.

18.   19.   20.   25.   26.
(22 B^I)   (20 B^I)   (9 B^I)   (11 A+7 B)   (3B)
I. 6.   II. 8.

⑫   13.   50.   51.   54.   55.
(50 B^I)   (11 B^I)   9 B^I   15 B^I   (12 B)   (7B)

I. 1.   II. 5.

⑨ (50 B^I plants)   10. (2 A+2 B)
I. 4.

III. 15.

5. (25 B^I plants)   7. (17 A+21 B)

I. 6.   II. 4.   II. 10.   II. 13.   III. 2.   I. 11.   I. 15.   II. 15.

18.   19.   20.   25.   26.
(22 B^I)   (20 B^I)   (9 B^I)   (11 A+7 B)   (3B)
I. 6.   II. 8.

⑫   13.   50.   51.   54.   55.
(50 B^I)   (11 B^I)   9 B^I 15 B^I (12 B)   (7B)

The buds in both reciprocal hybrids and all their descendants through five generations (1753 plants) showed the dominant red of *rubricalyx*. The significance of this important fact will be discussed later.

The F_1 of the reciprocal, *Oe. ammophila* $\times$ (*biennis* $\times$ *rubricalyx*), is very different. A single F_1 family (3·23) of 27 plants was grown. The two parental plants were the same individuals as in culture 1·23, and all later generations are descended from these

Fig. 3.—*Oe.* (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila*, F_1, showing oblique stems.

reciprocal crosses between the same two individuals. The F_1 rosettes had midribs which were white above, pale red below, the stems were erect (fig. 4) with long basal branches, and the leaves grey-green. The plants were of a single uniform type and showed the dominant red buds of *rubricalyx*. The rosettes were somewhat smaller than the reciprocal and the stems had less diffuse red pigmentation.

Oe. (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila*.

The F_2, F_3, F_4 and F_5 of these hybrids may now be briefly considered. The pedigree relationships of these families are shown in the charts figs. 1 and 2. Fig. 1 shows the F_1—F_5 families of *Oe.* (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila*, the number of each culture and the year in which it was grown being given, as well as the number of plants in a culture and the particular parent plant from which it was derived by selfing. Each of the 15 families, numbering in all 833 plants, usually represented a single uniform type, but the two F_1 families and one of the F_2 families showed segregation into twin types. The two types of the F_1 all had the oblique stems of *Oe. ammophila* and differed only in having light green or dark green leaves ; the three F_2 families were all derived from

selfed plants of the light green type. Two of these families gave identical, uniform offspring with erect stems and foliage resembling *rubricalyx*. The third family (24·25) showed a large majority (71) of the dark green (A) type, agreeing with the F_1 except in having erect stems; but **6 plants** belonged to the B type, with which they agreed in every particular, including the bent stem-tips. Although the A type have very narrow and the B type much broader leaves as seedlings (May 2), yet the later B rosettes (June 14) had leaves no broader than A, but longer and hence appearing relatively narrower than A. B was, therefore, the narrower-leaved type in the later stages of the rosette. The

Fig. 4.—*Oe. ammophila* × (*biennis* × *rubricalyx*) F_1.

Fig. 5.—*Oe* (*biennis* × *rubricalyx*) × *ammophila*, F_2, Type A.

type A plant (fig. 5) nearly resembles *rubricalyx* in shape and crinkling of the leaves, but differs in having much smaller flowers and hence agrees with the parental strain of *Oe. biennis* × *rubricalyx*. In type B, the habit and bent stem-tips closely resemble *ammophila*. We thus have in this family a segregation into two forms rather closely resembling the parental races.

Family 1·25 in fig. 1 differed from the family (4·24) from which it was derived, only in having slightly more zigzag stems, a sign of weakness. The other three F_3 families were all derived from light green (B) type plants of culture 24·25 and gave a uniform progeny of 122 plants which agreed with the parent type, except that the stem-tips showed no sign of bending. The F_4 and F_5 families, *i.e.*, 2·26, 3·26, 4·26, 2·27, 3·27 and 26·27, were all erect and uniform, the first three families belonging to type A, while

the last three differed in having slender unbranched stems. This may have been a result of their developing rather late in the season.

Thus in the five generations from *Oe.* (*biennis* × *rubricalyx*) × *ammophila*, the F_1 clearly inherited in full strength, if not in exaggerated form, the bent stems of the pollen parent, the two types represented in equal numbers differing only in leaf colour. In later generations only one F_2 family segregated, and later generations ($F_3 - F_5$) bred essentially true, except for minor differences. The very conspicuous bent stems of the F_1 were usually but slightly or variably represented in later generations, and most of the families were perfectly erect. The small number of the F_2 showing bent stem-tips indicates that this type was probably eliminated, either because of slower germination, lesser viability, or from some other cause, such as slower growth of the type of pollen tube which would produce it in fertilization.

Oe. ammophila × (*biennis* × *rubricalyx*).

Turning now to the reciprocal hybrid, *Oe. ammophila* × (*biennis* × *rubricalyx*), of which 53 families were grown in the five generations (fig. 2), it was in these families that the most extensive studies of flower-size inheritance have been made. As already explained, this F_1 hybrid is very different from the reciprocal (*cf.* figs. 3 and 4). Of the five F_2 families (1924), two showed segregation into twin types, while the other three were uniform and in agreement with the F_1. They differed from type B of the reciprocal hybrid in having narrower leaves, and were therefore called type B^1 (see fig. 6). The segregation of the other families was into type A, which agreed with type A of the reciprocal F_2 hybrid, and type B (fig. 7) which had *broader*, less crinkled leaves. As regards the A and B types, it should be pointed out that the former usually forms larger rosettes and is later in producing a stem, while the B type is earlier, the rosette stage being short or sometimes omitted altogether. In the A type the leaves are darker green, crinkled and broader at one stage of rosette development, becoming narrower than B at another. The B type has lighter green leaves, smooth and narrower than A, becoming broader later. Thus we have the curious result that they reverse their positions as regards the relative leaf-width, during the ontogeny. Both these types were very much like *rubricalyx*, except in having smaller flowers. (Both *Oe. biennis* and *Oe. ammophila* have much smaller flowers than *rubricalyx*.) In culture 10·24, one of the B plants had the stem bent laterally, as in *ammophila*.

The F_3 from this cross numbered six families, all grown in 1925. The first and last of these families were derived from families (6·24 and 10·24), which had segregated in F_2, and they now segregate again into two types A and B, resembling those of the reciprocal cross. The B type is now the more numerous, whereas in the reciprocal F_2, the A type largely predominated. The remaining four F_3 families, derived from families which were constant in F_2, remained true to the B^1 type, which is the same as the F_1 generation, and differs from B only in having constantly smaller and narrower

leaves. These four families were uniform, except that a few showed more or less tendency to express a peculiarity of *Oe. ammophila*, *i.e.*, the bent stem-tips.

In family 2·25, the plants were easily classified into their two types, whereas in 7·25 they were very difficult to classify, because the differences were less marked and the condition of the stems was variable, some being bent from near the base, some only near the tip, and some showing both a basal and an apical bend. Also some of the stems which were leaning at first afterwards became erect, or nearly so. This was observed in culture 2·25, where the plants were easily classified. The plants in this culture were also taller than in the reciprocal F_3 (1·25) grown alongside it.

Fig. 6.—*Oe. ammophila* × (*biennis* × *rubricalyx*), F_2, Type B¹.
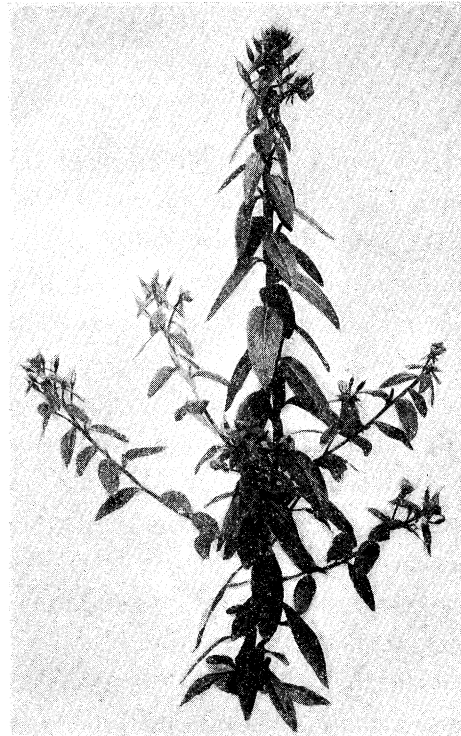
Fig 7.—*Oe. ammophila* × (*biennis* × *rubricalyx*), F_3, Type B.

The F_4 families numbered 31. Three of these bred true to type A, six to type B, twenty to type B¹, and two only segregated into A and B. Thus, again, these forms for the most part remain constant. Family 8·26 was produced by selfing 2·25 III. 3, a plant of type B. Similarly, the parent plants of culture 9·26, 45·27, 26·26, 54·27 and 55·27 were B. The other uniform F_4 families may also be assumed to have come in each case from a parent plant of the same type, although this was not actually recorded in every case. Thus the A family 44·27 was obtained by selfing 2·25 V.7, an A plant. Again in the families which segregated, both in F_4 and in F_3 it is always

a B plant which gives a family containing A and B, while A always breeds true. Thus family 15·26, which splits, was derived from a B[1] family (4·25), and family 25·26 from 7·25 I. 11, which was a B plant in a segregating family.

The F_5 families from this cross numbered ten, one of which bred true for A, one for B, and five for B[1]. The remaining three families were from reciprocal crosses between plants of the A and B types in family 15·26. Two of these families segregate in the F_1 into the parental types, while from the third a single B plant developed.

Conclusions.

From the five generations of cultures, the conclusion may be drawn that the F_1 of *Oe. ammophila* × (*biennis* × *rubricalyx*) called B[1] breeds true in many families, but splits in some F_2 families into twin types which agree in their main features with the F_2 types from the reciprocal, although the F_1 generations are so different. When the A and B types are crossed, they segregate into the parental types in F_1. The most striking fact concerning these triple hybrids is thus that they give rise to twin types which breed true in the main. The B type may also segregate the A type, but not *vice versa*. These and similar true-breeding hybrids in Oenothera appear to be explained in part by the linkage of their chromosomes during meiosis, to be described in the next section. From this we may draw the conclusion that it is possible that new species of Oenothera (and probably of some other genera) arise through crossing and remain constant through linkage of non-homologous chromosomes. How widespread this process may be in nature remains to be determined.

On the other hand, we have seen that certain families, such as 24·25 and 7·25, segregate into forms essentially resembling the two grandparental types. Thus the twin types produced appear to represent a partial and incomplete return to the original parents, or, in other words, a modification of the original types. These for the most part afterwards breed true, and they are also stable in the sense that crosses between them immediately segregate into the same two types that crossed.

As regards the inheritance of the dominant red bud colour of *Oe. rubricalyx*, it has already been pointed out that both the reciprocal triple hybrids here described as well as all of their descendants had red buds. This must mean that they are homozygous for the red factor, which differentiates *rubricalyx* from *rubrinervis*, and which arose as a dominant gene mutation in 1907 (see GATES 1915a). Now, as has already been stated, the particular F_5 family of *Oe. biennis* × *rubricalyx* which was used in making these crosses contained 12 R plants : 1 r. This approximates to the 15 : 1 ratio for two duplicate factors and suggests that the plant of this F_5 family was heterozygous for two factors for red. This is borne out by the fact that in earlier experiments with *rubricalyx* (GATES, 1915b), different families gave 15 : 1, 3 : 1, and also in some cases unexplained 5 : 1 ratios. The evidence indicates that a two-factor hypothesis rather than a differential rate of pollen tube growth is necessary to explain the results. Now

if the plant in question were heterozygous for two factors for red, then some of the plants in its offspring would be homozygous for two such factors.

As will be explained in the next section, the hybrids produced by crossing *Oe.* (*biennis* × *rubricalyx*) with *Oe. ammophila* show new chromosome pairing and linkages. There is some general evidence that this may be brought about in various cases by pairing between non-homologous chromosomes. Now if a plant from *Oe.* (*biennis* × *rubricalyx*) F_5 was homozygous for two R factors, then all its gametes would contain two chromosomes each bearing an R factor. If these two chromosomes paired with each other in the F_1 hybrid with *Oe. ammophila,* instead of finding mates among the *ammophila* chromosomes, then these F_1 hybrids would be homozygous for one pair of R factors, and all their descendants would breed true for red buds, as is actually the case. This hypothesis is the only one we have been able to devise which meets all the facts. If true, it would support the hypothesis already proposed on other grounds, that in some Oenothera hybrids a remating of some of the chromosomes takes place, so that non-homologous chromosomes become homologues. This would also support the suggestion made elsewhere (GATES, 1923) that the difference between homologous and non-homologous chromosomes is less sharp in Oenothera than in some other genera.

PART II.—CYTOLOGICAL.

Cytological Methods.

The greater part of the material which was examined cytologically was taken from the cultures grown during 1927, *i.e.*, from the F_5 generation. However, a small amount of material of the F_2 generation of the narrow-leaved type of *Oe. ammophila* × (*biennis* × *rubricalyx*) had been fixed in 1924 and was available for cytological study.

The flower buds were collected about noon on the warmest sunny days of July and August. The fixatives employed were ALLEN's modification of BOUIN's fluid and CARNOY's fluid. Ordinary BOUIN's fluid was used in a similar manner to ALLEN's modification, the mixture being heated to a temperature of 38° C. and being allowed to cool slowly whilst the buds were collected. To facilitate penetration of the anthers by the fixative the sepals were always removed and except in the case of CARNOY an exhaust pump was employed. The BOUIN and ALLEN's modified BOUIN were allowed to act for one to three hours. The best results were given by these two fixatives, the material fixed by CARNOY's method being on the whole but poorly preserved.

The fixed and dehydrated flower buds were cleared in xylol, embedded in paraffin wax and sectioned at 10–12μ. The most suitable stain had previously been found to be HAIDENHAIN's iron-alum hæmatoxylin and this was used principally throughout.

DESCRIPTION.

Cytological Study of the Reduction Division in the Pollen Mother-Cells.

Oe. ammophila × (biennis × rubricalyx).

In all, ten plants of this hybrid were studied cytologically. Four of these were of the narrow-leaved type and one of the broad-leaved type (see fig. 2). In 1926, the broad-leaved type was crossed with the narrow-leaved. This resulted in the segregation of the two parental types in the F_1 generation. Of this culture, five plants were examined, three broad-leaved (B) and two narrow-leaved (A). The examination of the meiotic divisions in the pollen mother-cells yielded identical results in all these plants. So far as can be observed the nuclear constitution and behaviour is precisely similar in the two twin hybrids and shows no variation from one generation to another, for the F_2 plant examined was in all details like those of the F_5 generation. Since, as shown in fig 2, families 9·24 and 7·27—9·27 were descended from different F_1 plants, it is highly probable that the F_1 showed the same condition, i.e., three pairs of chromosomes and a ring of eight, as explained later.

As the resting and earlier prophase stages are in all essentials similar to those already described for other Oenothera species (SHEFFIELD, 1927) it would be futile to give here a detailed account of these phases of meiotic division. The fine-meshed reticulum of the resting nucleus becomes gradually converted into a long, fine, single spireme, which is apparently continuous and is attached to the dark-staining endonucleolus. The thread shortens and thickens and quite soon it enters into synizesis. This first contraction lasts for a considerable period.

Throughout the prophase stages the nucleolus behaves similarly to those already described. It does, however, appear to be slightly vacuolate, becoming more so immediately prior to its ultimate disappearance. The vacuolation may be to some extent an artifact, as it is considerably increased by CARNOY's fixation. The endonucleolus was not seen in the CARNOY preparations, although it was regularly observed in all other suitably-stained preparations which showed the appropriate stages. It would appear that this nucleolar inclusion is destroyed by CARNOY's fixative, although a loop of the spireme remains apparently attached to the surface of the nucleolus.

Later Prophase Stages.

It is a considerably thickened thread which emerges at the loosening of the synizetic knot. During synizesis the biconvex nucleolus and the closely tangled spireme lie towards one side of the nuclear cavity, but when the knot begins to unfold, the chromatin thread again tends to spread throughout the cavity. The knot is, however, never completely disentangled, as is the case in many species, including Oe. rubrinervis (GATES, 1908), Oe. biennis and Oe. biennis sulfurea (CLELAND, 1926), Oe. muricata (CLELAND, 1926) and Oe. eriensis (SHEFFIELD, 1927). The period which elapses between synizesis

and the second contraction is very short, so that before the chromatin thread has become completely loosened it is tending to contract again. Thus, fig. 17* (Plate 89) illustrates the nearest approach to an open spireme which is ever reached. Occasionally it is possible to trace the course of the pachynema throughout almost its entire length during open spireme (*Oe. rubrinervis*) and sometimes segmentation may begin prior to second contraction as in *Oe. eriensis* and *Oe. Agari* (SHEFFIELD, 1927). Such forms offer a striking contrast to this hybrid where a relatively fine and consequently an extremely long pachytene thread enters the second contraction knot.

Second contraction is very prolonged in this hybrid. All the loops of the spireme are gradually drawn into the central knot and the thread continues to thicken. This mass of chromatin material is usually appressed to the nucleolus (fig. 18, Plate 89). The spireme is still connected to the endonucleolus, but the latter is often obscured by the chromatin knot. After a considerable period the loops of the knot become slightly loosened and it is then possible to see that the thickened spireme has become constricted at a number of points. Then, quite soon the nucleus is seen to contain a rather smaller knot of chromatin and three ring pairs of chromosomes (fig. 21).

It cannot be determined with any degree of certainty whether the pairs arise simultaneously or in rapid succession, but the latter would appear to be the case. Fig. 19 shows the spireme about to emerge from the second contraction knot and apparently a single pair has been cut off, but has remained interlinked with the main portion of the spireme. In fig. 20 two such bivalents appear and these again are interlocked with the spireme. It seems probable that the long loop which projects from the lower part of the knot and which could be seen to consist of two chromosomes will immediately give rise to a third ring pair. In fig. 21 a third bivalent has made its appearance, separate from what now remains of the second contraction knot. A number of nuclei showed a similar knot with either one, two or three bivalents lying free or linked to the main spireme. It cannot, however, be definitely concluded from this that the pairs are cut off successively, although this is most probably the case. It is possible that the three ring pairs are segmented from the spireme simultaneously, but that only one or two of them are set free. The other pairs or pair may become enmeshed in the knot and not set free until the latter is loosened. This is a point which it is impossible to determine with certainty, as the spireme is too closely entangled at the time of segmentation of the bivalents. We conclude that they may be cut off simultaneously, or if successively then in very rapid succession.

The chromatin knot now loosens and reveals a long continuous spireme which is becoming constricted into eight segments, each of which will form a chromosome (fig. 22). The chromosomes at this stage are large and of a spongy nature, but they rapidly condense (fig. 23). This condensation does not occur uniformly among all the chromosomes, so no significance can be attached to the size and shape which any of them assume

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* It has not been found necessary to reproduce all the photographs of plants, but the original numbering of the cytological figures is retained and they therefore begin with number 17.

about this time. Immediately after their segmentation from the main portion of the spireme the paired chromosomes may be interlinked either with the spireme or with each other. It is rare, however, in this hybrid to find such interlocking as late as diakinesis. Most usually the three bivalents take the form of rings, while the closed chain, which consists of eight univalents, is twisted and contorted to enable it to lie within the nuclear cavity (fig. 23). During diakinesis a number of fibrillæ arise in the cytoplasm immediately surrounding the nucleus. These radiate from several points around the nuclear membrane towards which they run tangentially (fig. 26). The nuclear membrane is rapidly becoming dissolved, and the nucleolus, which for some time has been becoming less well defined, finally disappears.

Heterotypic Division.

After the formation of the multipolar spindle the dissolution of the nuclear membrane proceeds rapidly. As soon as it has disappeared, the fibrillæ penetrate into the nuclear cavity (fig. 27). Gradually the multipolar spindle is converted into a bipolar structure. Meanwhile the chromosomes have come to occupy a central position, and the closed chain of eight univalents has become undulated, adjacent chromosomes lying in slightly different planes while alternate ones are practically co-planar. As the spindle becomes bipolar the adjacent chromosomes become attached to fibres passing to opposite poles, resulting in a zigzag arrangement when seen in profile (fig. 28). In a perfectly polar view of the spindle (fig. 29) the undulations in this ring can again easily be seen. Such a clear polar view of the metaphase where each chromosome is still attached to its neighbour is not often observed. The undulating form of the ring of eight univalents will not be seen clearly unless the section is cut absolutely perpendicular to the axis of the spindle, and the chances are very much against obtaining such a section. The three free pairs of chromosomes also take up their position about the equatorial region of the spindle (figs. 28 and 29).

At anaphase, the three bivalents assort in the usual way, each chromosome travelling to the opposite pole from its homologue. The delicate threads which connect the neighbouring univalents of the large ring are gradually drawn out, and consequently become finer. Eventually they break away, and the two groups of chromosomes are drawn apart, alternate ones passing to the same pole (fig. 30). Throughout the heterotypic division the chromosomes tend to become more and more compact, and they also vary considerably in shape according to the forces to which they are subjected. During diakinesis they are elongated, and usually more or less cylindrical; at metaphase they become V-shaped, and during the anaphase they tend to lose their rather angular form and later to become elliptical.

Fairly frequent instances of non-disjunction and of lagging chromosomes in anaphase are observed, and fragmentation of chromosomes on the spindle also occurs. These abnormalities and certain irregularities occasionally observed in diakinesis will be dealt with briefly later.

Oe. (*biennis* × *rubricalyx*) × *ammophila*.

In this case it was possible to examine material only of the narrow-leaved type. The reduction division was studied in the pollen mother-cells of three different plants in each of two different cultures (see fig. 1). There was no variation in the results given by the different plants, but the chromosome linkages were strikingly different from those in the reciprocal.

The earlier prophase stages are similar to those described for the reciprocal hybrid. The nucleolus is, however, considerably more vacuolated, becoming honey-combed during the later prophase stages. Here again the vacuolation appears to be increased and the endonucleolus destroyed by CARNOY fixation. With other fixatives a loop of the spireme was regularly found to be connected to the endonucleolus during the earlier prophase stages, and until the unfolding of the second contraction knot.

Later Prophase Stages.

As in the reciprocal, the period which elapses between synizesis and the second contraction is very brief. The spireme is at no time during this short interval completely loosened, as it is in several Oenothera species and in other genera such as Lilium (MOTTIER, 1907). After synizesis the knot becomes considerably loosened until eventually a number of loops project from a central mass of loosely entangled threads (fig. 37, Plate 90). The entire spireme then rapidly thickens and shortens, the central mass of threads becomes again tightly knotted, and the projecting loops are gradually drawn into the central mass (fig. 38). Eventually, when the height of second contraction is attained, the thread is many times its previous thickness, and all the loops have been completely drawn into the central mass, which lies against the nucleolus (fig. 39). Doubtless this approximation of the chromatin knot and the vacuolate nucleolus is due purely to the attachment which still persists between the spireme and the endonucleolus. Such a withdrawal of radiating loops of the spireme into the second contraction knot has been described as occurring in *Oe. franciscana* (CLELAND, 1922), *Oe. franciscana sulfurea* (CLELAND, 1924), *Oe. biennis* (LELIVELD, 1928, and CLELAND, 1924), *Oe. biennis sulfurea* (CLELAND, 1926), *Oe. muricata* (CLELAND, 1926) and *Oe. Lamarckiana* (LELIVELD, 1928). The number of radiating loops which can be seen is usually about 7—10; doubtless others would become visible if the nucleus could be viewed from the side. So far as could be ascertained there was no constancy in the number of these loops.

Second contraction begins relatively early in this hybrid and lasts for a considerable period, but in no case has the second contraction been found to continue for so long a time as does synizesis. Eventually the knot loosens slightly and a small ring is seen to have been cut off from the main portion of the spireme. This ring consists of a single pair of chromosomes (fig. 40). It may be interlinked with the rest of the spireme for a time after its segmentation but more usually it is set free at the time of origin. Such a precocious cutting-off of a single bivalent occurs also in *Oe. rubrinervis* (GATES, 1908),

and in *Oe. lata* two such ring pairs may be cut off thus early (GATES, 1907). The knot of chromatin material rapidly loosens and breaks away from the nucleolus. When the knot unfolds it is found to consist no longer of a continuous spireme but of six further ring pairs of chromosomes. Quite frequently the latter are all interlocked (figs. 41, 42). Very occasionally all the bivalents are free, as is the case at diakinesis in most plants and animals. Most frequently three or four of the pairs are free whilst the remainder are interlocked. During the later diakinesis stages, when the chromosomes have become considerably condensed, it is quite usual to find a number of the bivalents not forming rings but of which the two homologues are connected only at one extremity and lie end to end (figs. 44, 45).

It has usually been found that when ring pairs are interlocked at their time of origin, either with each other or with a ring of concatenated chromosomes, that they are later set free as in *Oe. franciscana* (CLELAND, 1922), *Oe. Lamarckiana* (HÅKANSSON, 1926), *Oe. rubricalyx*, *Oe. ammophila* (SHEFFIELD, 1927), and *Oe. ammophila* × (*biennis* × *rubricalyx*). Presumably one of the rings must break temporarily in order to free the pair; it must, however, close up again immediately, as in these species, although the rings may be interlinked in early diakinesis, they have usually been set free by the time the multipolar spindle is formed. Also it is unusual to find bivalents which do not take the form of rings or, if the nucleus is uncut, to find a chain of univalents which is not closed. In *Oe.* (*biennis* × *rubricalyx*) × *ammophila* an extraordinary amount of such interlocking occurs. The seven ring pairs of *Oe. grandfliora* (DAVIS, 1909) show similar behaviour, and the condition is described as persisting on to the spindle, although complicated figures of this kind are not drawn. In this hybrid, it would appear that when the connection between two homologues breaks in order that the bivalent may be set free, the affinity between the mates is not always sufficient to cause them to reunite. A little interlocking may be seen on the multipolar spindle (figs. 47, 48), but it is very rare as late as metaphase (fig. 49). Thus, during diakinesis and on the multipolar spindle until metaphase, the amount of interlinkage of bivalents steadily decreases, whilst the number of homologues which lie end to end instead of taking the form of small closed rings seems to increase. At the time of emergence from the second contraction knot all the bivalents form rings, whilst when metaphase is reached very few of them are joined at both ends.

Heterotypic Division.

The formation of the multipolar spindle, the dissolution of the nuclear membrane and the vacuolate nucleolus, and the derivation of the bipolar spindle occur in the usual manner. From the time of their formation the chromosomes condense, until when metaphase is reached they have but a fraction of their original bulk. When the bipolar spindle is formed, usually most of the bivalents come to lie near the equatorial region. However, they never form a regular equatorial plate. Usually some bivalents approach the centre of the spindle and the mates separate whilst other homologues are still joined

(fig. 50). This results as a rule in a very irregular metaphase and anaphase. Fig. 53 was the most nearly regular metaphase which was observed. In this case all the bivalents still have the form of rings, and hence were presumably all freed from one another at their time of origin. When the bivalents have still the ring form in metaphase each chromosome of the pair is usually V-shaped in side view ; when seen edge-on they appear elliptical (fig. 53). Those homologues which come to the metaphase connected only at one end do not assume the typical V-shape. The spindle fibres usually attach themselves to the chromosomes more or less in the central region, the homologues being drawn into a variety of peculiar shapes (figs. 50–52). Indications of a homotypic split may appear at this time.

As the metaphase is so irregular, it is obvious that the univalents comprising each separating group of chromosomes cannot travel simultaneously to the opposite poles. Once the homologues have broken apart they separate rapidly, but the univalents which will compose each daughter nucleus travel to the poles in a procession. In polar view (fig. 54) the univalents are arranged in what appears to be the normal manner for all Oenothera species, six members of each group being arranged in a circle in which the seventh is slightly eccentric.

Irregularities.

Diakinesis.

The conditions described as occurring constantly in diakinesis were observed in large numbers of pollen mother-cells. In a few exceptional cases divergences from the configurations described were observed. In the cross *Oe.* (*biennis* × *rubricalyx*) × *ammophila* no such variations were found ; in every pollen mother-cell in which the arrangement of the chromosomes could be interpreted with certainty, seven bivalents were always observed. Variation occurred only in the amount of interlocking between the ring pairs and in the number of bivalents which took the form of rings.

In the reciprocal cross, *Oe. ammophila* × (*biennis* × *rubricalyx*), a few pollen mother-cell nuclei were seen in which the amount of chromosome linkage occurring in meiosis was abnormal. In one case four bivalents and a closed chain of six chromosomes were present (fig. 24). A second abnormal configuration was observed in a nucleus which was unfortunately cut by the microtome knife and showed only thirteen chromosomes in one section. Two of these were joined and formed a ring, the remaining eleven univalents were joined end to end to form a single open chain, the two end chromosomes of which were brought close together and in fig. 25 appear to overlap. The fourteenth chromosome was found in the next section and occupied a position which indicated that it had once been in close proximity to the two end chromosomes of the chain. Probably then twelve chromosomes were linked into a ring whilst the remaining two were paired. The significance of such abnormalities has already been considered (SHEFFIELD 1927).

Heterotypic and Homotypic Mitosis.

An appreciable percentage of six-eight divisions has been observed in most Oenothera species which have been examined cytologically, and the genetical importance of these has been discussed (GATES, 1915; CLELAND, 1926; SHEFFIELD, 1927).

A number of cases of non-disjunction were observed in both the present hybrids. This abnormality may be due, where chromosome linkage occurs, to adjacent chromosomes of the chain passing to the same pole at anaphase. A metaphase which may result in such an abnormal segregation of chromosomes is illustrated in fig. 31. Two adjacent chromosomes are about to pass to the upper pole as is shown by the attachment of the spindle fibres. Another chromosome is similarly suspended between two which are about to pass to opposite poles. Such a condition may result in non-disjunction or double non-disjunction, according to whether this last chromosome passes to the upper or the lower pole.

Non-disjunction might also result from the failure of the members of a pair of chromosomes to separate at anaphase. In *Oe. (biennis × rubricalyx) × ammophila* especially the bivalents may be scattered over the spindle. It is conceivable that the two members of such a pair might be included within the same daughter nucleus. In this hybrid also a single pair of chromosomes may be obliquely or even transversely oriented on the spindle at metaphase (figs. 49, 52). It is possible that such pairs of chromosomes may not separate in the normal manner at anaphase.

Certain chromosomes have a tendency to lag in anaphase in both the hybrids. This tendency is much greater in *Oe. (biennis × rubricalyx) × ammophila* than it is in the reciprocal hybrid. In the latter, fairly regular anaphase figures (fig. 30) are often seen, whilst in the former the chromosomes are always very scattered, as in fig. 33. Such lagging of chromosomes in anaphase is undoubtedly a hybrid phenomenon. The fact that it is less marked in one of these hybrids than in the reciprocal is probably due to the chromosome linkage, which occurs in the one and which is absent from the other. The linked chromosomes must pass to the equator of the spindle simultaneously, the connections between them probably break away at approximately the same time and the chromosomes of each group derived from the ring would pass together to the pole. As will be seen later, linkage of chromosomes appears to result in much more regular cytological behaviour than does complete pairing.

Lagging of chromosomes in the heterotypic anaphase results quite frequently in one or more chromosomes being left out of the daughter nuclei. Chromosomes are also found occasionally to lag behind and fragment on the spindle during the heterotypic telophase (fig. 34)—these also are omitted from the daughter nuclei. Such chromosomes usually remain in the cytoplasm and eventually disintegrate. Occasionally, however, they form micro-nuclei. Lagging of chromosomes is observed also in the homotypic anaphase, especially in the hybrid *Oe. (biennis × rubricalyx) × ammophila,* resulting often in their omission from the grand-daughter nuclei. Such chromosomes probably usually disintegrate, but these also may form micro-nuclei. Those nuclei which do not receive

their full quota of chromosomes after either heterotypic or homotypic mitosis continue to develop in the usual way.

Following are given the estimated irregularities occurring in heterotypic and homotypic mitosis in the two present hybrids :—

TABLE II

—	Oe. ammophila × (biennis × rubricalyx)	Oe. (biennis × rubricalyx) × ammophila
No. of 7-7 divisions (counts made in homotypic metaphase)	331	191
No. of 6–8 divisions (counts made in homotypic metaphase)	7	3
∴ Ratio of 6-8 to normal 7-7 divisions	2 per cent.	1·5 per cent.
No. of cells showing normal interkinesis	178	408
No. of cells in interkinesis with one or more chromosomes left out of either daughter nucleus	6	57
∴ Proportion of cells in which one or more chromosomes are omitted from either daughter nucleus at interkinesis	3 per cent.	12 per cent.
No. of cells in homotypic telophase	251	276
No. of cells in homotypic telophase where some chromosomes have been left out of the grand-daughter nuclei	2	15
∴ Proportion of cells in homotypic telophase in which all chromosomes are not included in the grand-daughter nuclei	0·8 per cent.	5 per cent.

The percentage of irregularities given in the above table are in some cases based on comparatively low numbers of observations and consequently may not be accurate. They do, however, show clearly that a much larger percentage of irregularities is characteristic of *Oe. (biennis × rubricalyx) × ammophila* than usually occurs in the reciprocal hybrid. The proportion of cells in which chromosomes are omitted from the nuclei, which are reorganized after heterotypic and after homotypic mitosis, is much more considerable when *Oe. ammophila* is pollen parent and *Oe. (biennis × rubricalyx)* the seed parent than in the reciprocal cross. The ratio of 6–8 divisions to the normal 7–7 is apparently less in the former case than in the latter.

It must, however, be remembered that these counts were made in homotypic metaphase, those cells in which the total number of chromosomes in the two daughter nuclei were less than fourteen being disregarded. A number of 6–8 divisions were probably thus eliminated. If a chromosome or chromosomes were left out of the daughter nuclei in interkinesis, and disintegrated before homotypic metaphase, then the pollen mother-cell containing them would have been disregarded when the counts were made.

It must not, therefore, be assumed from these figures that 6–8 divisions are less

frequent in the hybrid showing complete pairing of chromosomes than in that which shows linkage. The estimated proportion of irregular homotypic divisions is probably greater than the actual, as certain of the micro-nuclei and chromosomes observed in the cytoplasm may have been left out in the heterotypic division and have persisted until the homotypic telophase.

Studies of the somatic mitoses in the petals have confirmed the earlier results (GATES, 1912; GATES and THOMAS, 1914), both as regards the details of the process and also the variability in the number of chromosome pairs observed in any one plant. Figs. 35 and 36, from mitoses in the young petals of *Oe. ammophila* × (*biennis* × *rubricalyx*), showed in one case no obvious pairing and in the other a large amount of apparent pairing, while the meiotic chromosomes are constantly in three pairs and a ring of eight. Figs. 55 and 56 are taken from a single plant of the reciprocal hybrid. In one there appears to be but little pairing, while the other shows several probable pairs. Hence, the amount of somatic pairing appears to be variable in the individual and independent of the arrangement taken up during meiosis.

DISCUSSION.

Pairing and Linkage of Chromosomes.

Several notable facts regarding the pairing and linkage of chromosomes in these hybrids have been brought to light. The first is with regard to the differences found to exist between the two reciprocal hybrids. Although the chromosomal constitution of these two hybrids is the same, yet differences in morphological structure and cytological behaviour are evident. When *Oe. ammophila* is the pollen parent, each chromosome of the two groups which are brought together finds a mate, with which it becomes paired. However, in the case of the reciprocal cross, when what must be regarded as virtually the same chromosomes are brought together, only six of them pair, the other eight remaining linked in a ring. It would seem then that some important part must be played by the cytoplasm, which is derived from *Oe.* (*biennis* × *rubricalyx*) in one cross and from *Oe. ammophila* in the other. When the cytoplasm of the egg is contributed by the constant hybrid *Oe.* (*biennis* × *rubricalyx*) 100 per cent. pairing occurs, but when *Oe. ammophila* is seed parent then eight of the chromosomes remain linked.

These linkages have only been actually observed to begin with diakinesis in the pollen mother-cells, but it is possible that the greater attraction which leads to the formation, *e.g.*, of the three pairs in *Oe. ammophila* × (*biennis* × *rubricalyx*) expressed itself in a special arrangement of the chromosomes in the fertilized egg from which the hybrid originally developed. A preliminary investigation, however, has yielded no positive evidence of a difference in the amount of somatic pairing in the reciprocal hybrids, but the number of chromosome pairs appears to vary in different somatic mitoses of the same plant. This subject, as well as the size variations of the chromosomes in the metaphase plate, is worthy of further investigation.

But when meiosis occurs the maternal and paternal chromosomes pair in a definite way. In *Oe. ammophila* $\times$ (*biennis* $\times$ *rubricalyx*) i.e., the haploid chromosomes of the two parents in *ammophila* cytoplasm, three pairs are formed, the other eight remaining connected in a ring; but when, in the reciprocal hybrid, the same two haploid sets of chromosomes are in the cytoplasm of the constant hybrid *Oe. biennis* $\times$ *rubricalyx*, then all the chromosomes are able to pair and seven pairs are formed. The conclusion seems inevitable that the cytoplasm plays a part in determining what pairing shall take place; in other words, it must influence the attractions between the chromosomes and the distribution of chromosomes which will take place in the reduction division. This in itself would necessitate a departure from universal Mendelian behaviour in these hybrids.

In the case of the twin hybrids of *Oe. ammophila* $\times$ (*biennis* $\times$ *rubricalyx*) although morphological differences are evident there are no apparent differences in the cytological behaviour. As individual chromosomes cannot be identified, it is possible that the linkage and pairing which occurs is between different chromosomes in the two cases—it being only a coincidence that the proportion of pairing is the same in both types. This, however, does not seem probable, and since the twin types both have the same cytoplasm, is not to be expected. The configuration observed is known to have persisted through five generations. The F_2 material studied was not all directly descended from the F_2 plant which was examined cytologically (see diagram of cultures in fig. 2), although similar results were obtained. The configuration must therefore in each case have been inherited from the original F_1 culture ($3 \cdot 23$), the chromosomes having so arranged themselves when first brought together. The F_1 generation, numbering 27 plants, contained only the narrow-leaved type, the broad being split off in various cultures in later generations. The two types may both occur in one culture, and when crossed together they immediately segregate out again in the F_1 generation. Some plants of both types observed in this way were examined from culture $9 \cdot 27$ and still the usual chromosome configuration was found.

It has been suggested (EMERSON, 1924) that where there is no pairing of chromosomes there are no homologous chromosomes. It is obvious that the chromosomes must occupy definite positions in the ring, as adjacent ones always separate in anaphase. If each chromosome does not have a definite position then innumerable genetic variations would arise. If homologues are not present it is difficult to conceive any reason why the chromosomes should be arranged in a definite manner.

Furthermore, in the case of *Oe. ammophila* $\times$ (*biennis* $\times$ *rubricalyx*) linkage of chromosomes is not due to absence of homologues, as in the reciprocal cross one hundred per cent. pairing occurs regularly. It is possible that there is little difference in the attraction existing between some homologous and non-homologous chromosomes and that this balance is upset by the presence of the cytoplasm of the maternal parent.

The occurrence of seven pairs in *Oe.* (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila* is of interest from another point of view. Previous writers have regarded the presence of

complete pairing as a proof that the species was homozygous ; thus Davis (1909) for *Oe. grandiflora*, and Cleland (1922) for *Oe. franciscana*. But complete pairing of chromosomes is shown in the above triple hybrid which one cannot reasonably assume to be a homozygous form. This indicates that pairing depends upon something more than the attraction between homologous chromosomes of similar genetic constitution.

Several papers recently published deal with the question of synapsis of the chromosomes in the genus Oenothera. Although it cannot be claimed that this work throws any new light on the problem, a word on the subject would not be out of place here. The earliest workers on meiosis in Oenothera were able to trace a spireme, which gradually shortened and thickened, and finally became constricted into a number of parts equal to the number of chromosomes and arranged end to end (Gates, Davis). Also in the earlier prophase stages no evidence of parallelism of threads more than would be a result of chance was observed. These observations have since been confirmed by a number of workers and for a long time it was generally conceded that the mode of pairing of the chromosomes in Oenothera was telosynaptic.

Following a study of heterotypic nuclear division in *Oe. Lamarckiana*, Boedijn (1924) put forward the view of parasynapsis in Oenothera. However, his observations have since been refuted by other workers (Håkansson, 1926 ; Cleland, 1922–26). By comparison with the related genus Eucharidium, Schwemmle (1926) is inclined to interpret Oenothera as being parasynaptic. Later Kihara (1927) put forward an interesting theory. He gives a parasynaptic account of meiosis in *Rumex acetosella*, but describes in diakinesis a ring of six linked chromosomes similar to those which occur in some Oenothera species. This ring is believed to arise from the double spireme in one of two ways—the " doppelketten " and the " radial." In the former case the end chromosomes of the two halves of a double spireme become joined at their outer ends, the two parts of the double thread then open out and so form a ring. In the latter case, a number of pairs of chromosomes, in which the two halves lie parallel, radiate from a central area. Each chromosome is attached at one end to its homologue and at the other to one chromosome of the neighbouring pair. These conditions are described in *Rumex acetosella*, and Kihara suggests that in Oenothera the rings may arise by some such " radial " arrangement. No cytological evidence in support of such an assumption was forthcoming.

Boedijn and Leliveld claim to have observed parallel threads in prophase, but these have been seen by no other workers. The only evidence which now supports it in any way is the condition described by Leliveld of seven loops (the two arms of each being parallel) which project from the second contraction knot. A similar condition has been observed by Cleland in several species, but there was no constancy in the number of loops nor were the two arms of each loop parallel. Fig. 38 illustrates a similar condition, and was frequently observed in *Oe. (biennis × rubricalyx) × ammophila*, but the loops varied greatly in size. The number also was in no way constant, but there were usually more than seven, and the arms were

not usually conspicuously parallel. Such a stage can in no way be considered as critical and its possible significance has been greatly over-rated. Moreover, in some species a single continuous spireme, which is constricted into fourteen parts lying end to end, can be seen during open spireme. In Lathyrus, on the other hand (LATTER, 1926), which is clearly telosynaptic, the number of loops corresponds with the haploid number of chromosomes.

In those species of Oenothera in which all fourteen chromosomes do not remain united throughout diakinesis, the segmentation of the spireme undoubtedly occurs during the second contraction. The *raison d'être* of the second contraction is thus probably to bring the ends of appropriate chromosomes into close proximity with one another, so that the spireme may break in two places, then fuse again differently, and so cut off a ring. The time occupied by the second contraction varies greatly in different species. It seems to be accomplished most rapidly where no segmentation occurs, as in *Oe. eriensis* and *Oe. novæ-scotiæ*, and is much more prolonged where pairing is complete as in *Oe.* (*biennis* × *rubricalyx*) × *ammophila* and in *Oe. grandiflora* (DAVIS, 1909). In those species in which only partial segmentation occurs, the time occupied by second contraction varies, but it is never accomplished so rapidly as it is in, for example, *Oe. eriensis*, and is not usually so prolonged as in *Oe.* (*biennis* × *rubricalyx*) × *ammophila*.

As regards the linkages in the parents of these reciprocal hybrids, *Oe. ammophila* is known (SHEFFIELD, 1927) to have one pair and a ring of twelve. *Oe. biennis* has a ring of eight and a ring of six (CLELAND, 1926), while *Oe. rubricalyx* has four pairs and a ring of six (SHEFFIELD, 1927), but, unfortunately, the pairing of the chromosomes in *Oe. biennis* × *rubricalyx* has not been determined. The F_5 of a practically constant race of this hybrid was used in the above crosses, and it may be safely assumed that whatever were the linkages in *Oe. biennis* × *rubricalyx*, they were as nearly constant as in the present triple hybrids.

Finally, it appears that the production in F_1 of true-breeding hybrid types, which is such a characteristic feature of the genus Oenothera, as shown by the abundant work of DE VRIES (1913) and others, is to be explained through the occurrence of chromosome linkage, which prevents free assortment of the chromosome pairs, and hence of the differential characters. Since linkage differences are equally characteristic of the wild species and the mutations arising from them in controlled experiments, we arrive at a picture of the evolution of the genus Oenothera, as having taken place through germinal changes (mutations) of various kinds (see GATES, 1915a) arising in a succession of species which are of natural hybrid origin but which in the main breed true because of their persistent chromosome linkages in meiosis. Certain aspects of this subject have been discussed elsewhere (GATES, 1928a, 1928b). Here it is only necessary to point out that in the existence of wild species and artificial hybrids which appear to breed true because of chromosome linkage we have a new evolutionary phenomenon which may be of much significance for the student of the origin of species.

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SUMMARY.

An account is given of five generations of hybrids from *Oenothera* (*biennis* × *rubricalyx*) × *ammophila*, and *Oe. ammophila* × (*biennis* × *rubricalyx*), and their cytological peculiarities. The chromosome linkages appear to be a means of explaining some of the genetical behaviour observed in these and similar hybrids. The reciprocal F_1 hybrids are very different. They are patroclinous, *Oe.* (*biennis* × *rubricalyx*) × *ammophila* especially showing the peculiar leaning stems and bent stem-tips of *Oe. ammophila*. The *rubricalyx* character—an excess of anthocyanin in various parts—is dominant, however, as in all crosses of the mutant *Oe. rubricalyx* hitherto made.

The F_1 of *Oe.* (*biennis* × *rubricalyx*) × *ammophila* contained two types which differed only in having light or dark green leaves. In F_2, two families were erect, uniform, and resembled *rubricalyx*, while the third family segregated into two types : A, which agreed with the dark green F_1 plants except in having erect stems ; and B, having light green leaves and bent stems. These types in F_3, F_4 and F_5 bred true in essentials.

The F_1 of *Oe. ammophila* × (*biennis* × *rubricalyx*) contained a single type (B^1), which was erect, but had the grey-green leaves of *Oe. ammophila*. In F_2, three families bred true and two segregated into the types A and B, which resembled the original parents of the cross. In later generations the A type bred true, B plants produced B and A, and B^1 families bred true. Further, when the A and B types are crossed together they produce an F_1 of A and B plants.

The strain of *Oe.* (*biennis* × *rubricalyx*) F_5, which was used in making the crosses, was probably heterozygous for two factors R for *rubricalyx* pigmentation. The descendants from the reciprocal hybrids (1753 plants) showed red (R) buds without exception. The explanation suggested is that the plant used in these crosses was homozygous for two R factors, and in the hybrid the two chromosomes carrying R paired with each other. There are general grounds for assuming that non-homologous chromosomes may form a pair in certain Oenothera hybrids. On such an hypothesis it may be explained how new heterozygous species, such as are characteristic of Oenothera, could be formed by crossing, and at the same time the two complexes of which they consist be non-viable in the homozygous condition.

The cytological results of chief significance concern the linkages between chromosomes, which are characteristic of Oenothera during diakinesis and the heterotypic metaphase. These linkages are found to be unlike in the reciprocal hybrids. In *Oe. ammophila* × (*biennis* × *rubricalyx*) the spireme segments in diakinesis into three free pairs of chromosomes and a ring of eight. The three pairs are probably cut off from the spireme in rapid succession. On the heterotypic spindle, the closed ring of eight chromosomes becomes undulated, while the pairs orient themselves in the usual way. Non-disjunction

and lagging of chromosomes at this time are not infrequent. Ten plants belonging to F_2 and F_5 families all showed identical conditions, indicating that this arrangement of pairing was taken up in the F_1 plants.

In *Oe.* (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila* there are, on the contrary, seven chromosome ring pairs. These are frequently interlinked with each other during diakinesis. Such interlocked ring pairs have to open out in order to separate from each other, and they then remain open. As a result, variously-shaped pairs of chromosomes appear on the heterotypic spindle. Lagging of chromosomes and other irregularities are much more frequent in this hybrid than in the reciprocal. This difference in the pairing of chromosomes in reciprocal hybrids, one may suppose to be due to the influence of the cytoplasm contributed by the egg-cell, but it only shows itself clearly at the time of meiosis. No evidence has been obtained of a corresponding amount of pairing in somatic divisions, where the evidence indicates that the closeness of pairing varies within a plant and does not correspond to the paired arrangement found in diakinesis.

The fact that the triple hybrid *Oe.* (*biennis* $\times$ *rubricalyx*) $\times$ *ammophila* has all its chromosomes paired, makes it clear that complete pairing cannot be regarded as a sign of the homozygous condition, as has been assumed by some previous writers. On the other hand, it is not certain that the hybrid with seven free pairs of chromosomes shows a greater amount of genetic segregation than the reciprocal with only three pairs and the remainder combined into a ring. It may be held as a tentative hypothesis that the linkage of chromosomes in Oenothera has arisen between non-homologous chromosomes as a result of crossing. The formation of permanent heterozygous species, the components of which are non-viable in the homozygous condition, as in various Oenothera species, can be accounted for through the mating of certain non-homologous chromosomes to form a pair in the F_1 of a cross.

The very characteristic and relatively fixed arrangement of the meiotic chromosomes, which is now known in many species, hybrids and mutations of Oenothera, must have some general significance, especially if, as appears probable, the positions of the various chromosomes in the spireme are fixed. Future work will determine the genetic significance of these linkages, but it appears probable that a relation exists between the linkage of chromosomes and the genetic linkage, which is such a characteristic feature of the genus Oenothera.

REFERENCES CITED.

BOEDIJN, K., 1924. " Die typische und heterotypische Kernteilung der Oenotheren." ' Zeits. f. Zellen- u. Gewebelehre,' vol. 1, pp. 265–277, figs. 17.

CLELAND, R. E., 1922. " The reduction divisions in the pollen mother-cells of *Oenothera franciscana*." ' Amer. Journ. Botany,' vol. 9, pp. 391–413, Plates 3.

—— 1924. " Meiosis in pollen mother-cells of *Oenothera franciscana sulfurea*." ' Bot. Gazette,' vol. 77, pp. 149–168, Plates 2.

CLELAND, R. E., 1926a. " Cytological study of meiosis in anthers of *Oenothera muricata*." ' Bot. Gazette,' vol. 82, pp. 55–70, Plates 4.

—— 1926b. " Meiosis in the pollen mother-cells of *Oenothera biennis* and *Oenothera biennis sulfurea*." ' Genetics,' vol. 11, pp. 127–162, Plates 2, figs. 7.

DAVIS, B. M., 1909. " Cytological studies on Oenothera. I. Pollen development of *Oenothera grandiflora*." ' Ann. Botany,' vol. 23, pp. 551–571, Plates 2.

—— 1910. II. " The reduction divisions of *Oenothera biennis*." ' Ann. Botany,' vol. 24, pp. 631–651, Plates 2.

—— 1911. III. " A comparison of the reduction divisions of *Oenothera Lamarckiana* and *Oe. gigas*." ' Ann. Botany,' vol. 25, pp. 941–974, Plates 3.

EMERSON, S. H., 1924. " The absence of chromosome pairing during meiosis in *Oenothera biennis*." *Papers Mich. Acad. of Sci. Art and Letters*, vol. 4, pp 111–114, Plates 3.

GATES, R. R., 1907. " Pollen development in hybrids of *Oenothera lata* × *Oe. Lamarckiana*, and its relation to mutation." ' Bot. Gazette,' vol. 43, pp. 81–115, Plates 3.

—— 1908. " A study of reduction in *Oenothera rubrinervis*." ' Bot. Gazette,' vol. 46, pp. 1–34, Plates 3.

—— 1910. " Abnormalities in Oenothera." ' 21st Rept. Mo. Bot. Garden,' vol. 21, pp. 175–184, Plates 3.

—— 1912. " Somatic mitoses in Oenothera." ' Ann. Botany,' vol. 26, pp. 993–1010, Plate 1.

—— 1913. " A contribution to a knowledge of the mutating Oenotheras." ' Trans. Linnean Soc., 2nd Ser. Botany,' vol. 8, pp. 1–67, Plates 6.

GATES, R. R. and THOMAS, N., 1914. " A cytological study of *Oenothera* mut. *lata* and *Oe*. mut. *semilata* in relation to mutation." ' Quart. Journ. Micro. Sci.,' vol. 59, pp. 523–571, Plates 3, figs. 4.

GATES, R. R., 1915a. " The mutation factor in evolution, with particular reference to Oenothera." London : (Macmillan), pp. 353, figs. 114.

—— 1915b. " On successive duplicate mutations." ' Biol. Bull.,' vol. 29, pp. 204–220.

—— 1917. " Vegetative segregation in a hybrid race." ' Journ. Genetics,' vol. 6, pp. 237–252, Plate 1.

—— 1923. " A peculiar type of variability in plants." ' Journ. Genetics,' vol. 13, pp. 13–45, figs. 24.

—— 1928a. " The relations of cytology to genetics in Oenothera." *Verh. V. Internat. Kongress. f. Vererbungswiss. Berlin*, 1927, vol. 1, pp. 749–758.

—— 1928b. " The cytology of Oenothera." ' Bibliographia Genetica,' vol. 4, pp. 401–492, figs. 39.

HÅKANSSON, A., 1926. " Über das Verhalten der Chromosomen bei der heterotypischen Teilung schwedischer *Oenothera Lamarckiana* und einiger ihrer Mutanten und Bastarde." ' Hereditas,' vol. 8, pp. 255–304, Plate 1, figs. 5.

Kihara, H., 1927. " Über das Verhalten der " end to end " gebundenen Chromosomen von *Rumex acetosella* und *Oenothera biennis* während der heterotypischen Kernteilung." ' Jahrb. f. wiss. Bot.,' vol. 66, pp. 429–460, figs. 89.

Latter, J., 1926. " The pollen development of *Lathyrus odoratus*." ' Ann. Botany,' vol. 40, pp. 277–313, Plates 3.

Leliveld, J. A., 1928. " Some remarks on the cytology of Oenothera." ' Rec. Trav. Bot. Néerlandais,' vol. 25a, pp. 237–243, figs. 6.

Mottier, D. M., 1907. " The development of the heterotypic chromosomes in pollen mother-cells." ' Ann. Botany,' vol. 21, pp. 309–347, Plates 2.

Oehlkers, F., 1926. " Erblichkeit und Zytologie einiger Kreuzungen mit *Oenothera strigosa*. (Vererbungsversuche an Oenotheren IV)." ' Jahrb. f. wissens. Bot.,' vol. 65, pp. 401–446, figs. 7.

Renner, O., 1925. " Untersuchungen über die faktorielle Konstitution einiger komplexheterozygotischer Oenotheren." ' Bibliotheca Genetica,' Leipzig, vol. 9, pp. 1–168, figs. 58.

Schwemmle, J., 1926. " Vergleichend zytologische Untersuchungen an Onagraceen. II. Die Reduktionsteilung von *Eucharidium concinnum*." ' Jahrb. f. wissens. Bot.,' vol. 65, pp. 778–818, Plates 10.

Sheffield, F. M. L., 1927. " Cytological studies of certain meiotic stages in Oenothera." ' Ann. Botany,' vol. 41, pp. 779–816, Plates 3, figs. 3.

Sinotô, Y., 1927. " Microsporogenesis in *Oenothera sinuata L*." ' Bot. Mag. Tokyo,' vol. 41, pp. 225–234, Plate 1.

de Vries, H., 1907. " On twin hybrids." ' Bot. Gazette,' vol. 44, pp. 401–407.

—— 1913. " Gruppenweise Artbildung, unter spezieller Berücksichtigung der Gattung Oenothera." Berlin (Bornträger), pp. 365, Plates 22, figs. 121.

EXPLANATION OF PLATES.

All figures were drawn with aid of a camera lucida at table level, and have been reproduced without reduction. A 2-mm. imm. Zeiss apochromatic objective (N.A. 1.4) and Zeiss comp. oc. 18 were always used. Magnification × 2,950. The chromosomes are shaded according to the plane in which they lie—those in top focus being black, whilst the lower ones are paler.

PLATE 89.

Oenothera ammophila × (*biennis* × *rubricalyx*).

Meiotic division. (Figs. 17–34.)

The cytological conditions in the broad-leaved and narrow-leaved types were precisely alike.

Fig. 17.—Narrow-leaved type. Open spireme. The pachynema is still very long and fine. Although the synizetic knot has not completely loosened, second contraction is imminent.
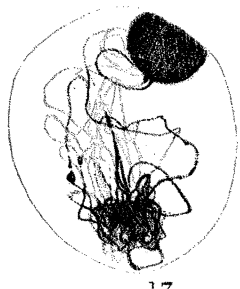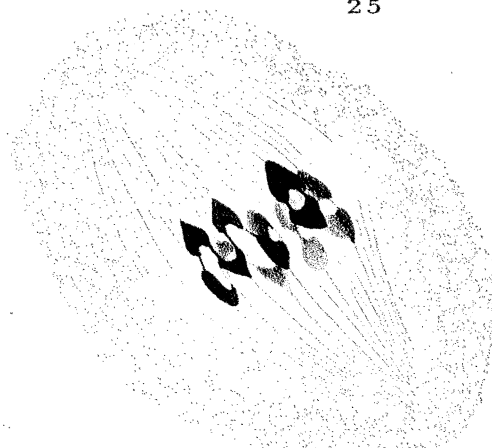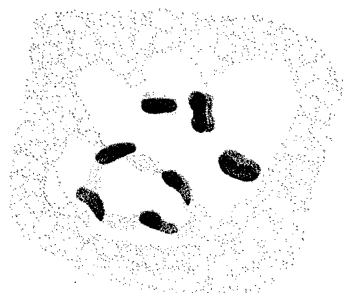
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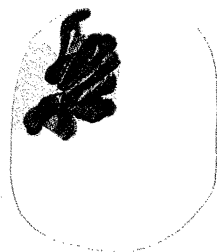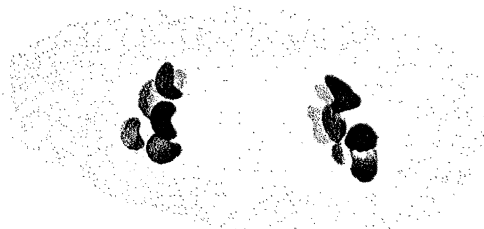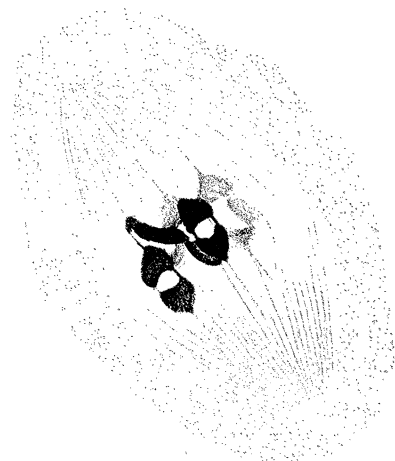
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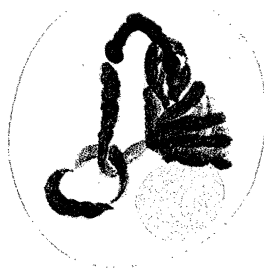
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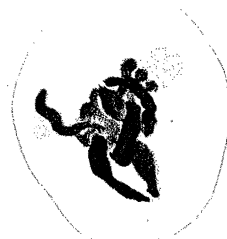
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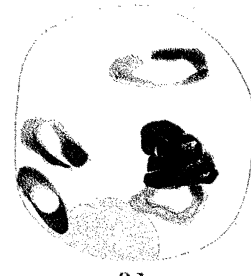
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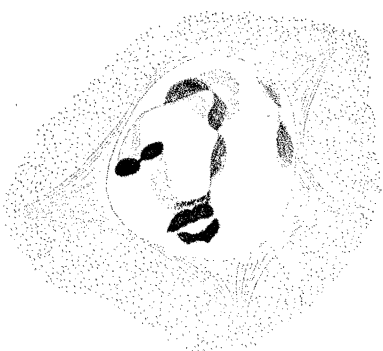
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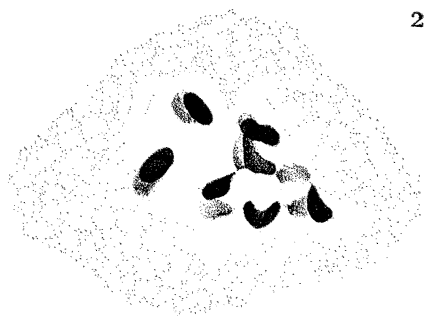
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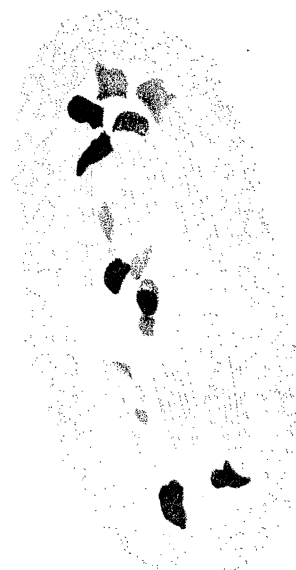
FIG. 18.—Broad-leaved type. Second contraction. The spireme has thickened considerably, and is again tightly entangled. It is connected to the pale-staining biconvex nucleolus.

FIG. 19.—Broad-leaved type. The second contraction knot has loosened slightly, and the thread can be seen to have become constricted into a number of parts. A single ring pair has been cut off from the spireme, but the two are interlocked.

FIG. 20.—Broad-leaved type. Two ring pairs of chromosomes have been segmented off from the spireme and are interlinked with it. A third loop, consisting of two chromosomes and projecting from the central knot, may give rise to a third bivalent.

FIG. 21.—Broad-leaved type. Three ring pairs of chromosomes have been set free from the second contraction knot.

FIG. 22.—Broad-leaved type. The second contraction knot has loosened, three ring pairs of chromosomes are now visible, and a large twisted ring of chromatin which has been constricted into eight parts. One bivalent is interlinked with the ring and with another pair.

FIG. 23.—Narrow-leaved type. Diakinesis. The nucleus contains a ring of eight chromosomes and three free ring pairs. The nucleolus, which is in bottom focus, and consequently appears spherical, has become very indistinct. The chromosomes are losing their spongy nature.

FIG. 24.—Broad-leaved type. Abnormal diakinesis. Six of the chromosomes are linked to form a ring, the remaining eight are paired.

FIG. 25.—Narrow-leaved type. Abnormal diakinesis. The nucleus is cut, and this section contains only thirteen chromosomes, eleven of which form a chain, while two form a small ring. The fourteenth chromosome is in the adjacent section, and occupies a position indicating that it was once in close proximity to the two overlapping ends of the chain.

FIG. 26.—Narrow-leaved type. Formation of the multipolar spindle. Chromosomes arranged in a contorted ring of eight with three free bivalents. The nucleolus has disappeared.

FIG. 27.—Narrow-leaved type. Multipolar spindle. The eight chromosomes of the ring have become slightly undulated, preparatory to assuming their appropriate positions on the bipolar spindle.

FIG. 28.—Narrow-leaved type. Metaphase. The spindle has become bipolar, and the chromosomes are arranged near the equatorial region. Adjacent chromosomes of the ring of eight have become attached to fibres from opposite poles.

FIG. 29.—Narrow-leaved type. Polar view of metaphase. The ring of chromosomes is undulated owing to the adjacent chromosomes being attached to fibres from opposite poles. The three bivalents lie towards one side of the spindle.

FIG. 30.—Broad-leaved type. Anaphase.

FIG. 31.—Broad-leaved type. Abnormal metaphase. The nucleus is cut, twelve chromosomes only being present. The chromosomes of the ring are not orientated in the usual way (see text).

FIG. 32.—Broad-leaved type. Abnormal anaphase. The connection between two of the chromosomes has so far failed to break away.

FIG. 33.—Broad-leaved type. Abnormal anaphase. Certain of the chromosomes are lagging.

FIG. 34.—Broad-leaved type. Abnormal anaphase. Five of the chromosomes have fragmented.

Mitotic Division. (Figs. 35–36.)

FIG. 35.—Metaphase in polar view. The chromosomes are scattered in the equatorial plate.

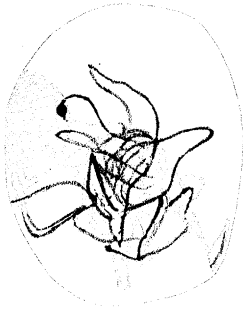FIG. 36.—Polar view of metaphase. The chromosomes are apparently mostly paired.

PLATE 90.

Oenothera (*biennis* × *rubricalyx*) × *ammophila.*

Meiotic Division.   (Figs. 37–54.)

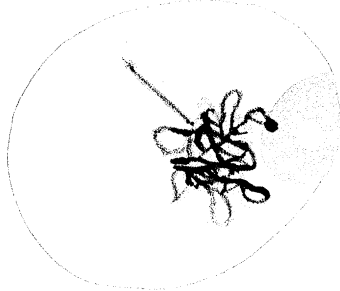All figures are drawn from the narrow-leaved type, the broad-leaved not having been examined.

Fig. 37.—Open spireme. Numerous looped threads project from a central tangle. One of these loops is attached to the endonucleolus.

Fig. 38.—Beginning of second contraction. The pachytene thread has shortened and thickened, the loops being drawn into the central knot. One loop is attached to the endonucleolus which lies at the periphery of the vacuolate nucleolus.

Fig. 39.—The acme of second contraction. All the loops have now been drawn into the knot, which lies against the nucleolus. The spireme is considerably thickened.

Fig. 40.—The second contraction knot has loosened slightly. A single ring pair of chromosomes has been cut off but has remained interlinked with the spireme. The nucleolus is very vacuolate, some of the vacuoles containing crystalloid-like bodies.

Fig. 41.—The loosening of the second contraction knot. The pair of chromosomes which was cut off precociously has been set free, the other six bivalents, each of which forms a ring, are interlocked.

Fig. 42.—Diakinesis. Six of the ring pairs are interlocked whilst the seventh is free.

Fig. 43.—Diakinesis. Three of the ring pairs are free whilst the remainder are interlocked.

Fig. 44.—Diakinesis. In two cases the chromosomes constituting the bivalents are joined only at one end and consequently do not form rings. Three of the ring pairs are free whilst two are interlocked.

Fig. 45.—Diakinesis. Only three of the bivalents form rings, two of which are interlinked. In the case of the other bivalents the homologues lie end to end.

Fig. 46.—Formation of the multipolar spindle. Four of the ring pairs are interlinked to form a chain, whilst the fifth is free. The remaining bivalents do not form rings.

Fig. 47.—Multipolar spindle. Six of the ring pairs are still interlocked.

Fig. 48.—Multipolar spindle. Interlocking between the ring pairs still occurs.

Fig. 49.—Metaphase. Two ring pairs are still linked together. This condition is unusual at such a late stage. Two of the pairs lie obliquely on the spindle.

Fig. 50.—Metaphase. Some pairs are lying at the central region of the spindle whilst some homologues have already separated.

Fig. 51.—Metaphase. The chromosomes composing the bivalents which retained the ring form are now V-shaped. The other chromosomes now vary greatly in shape.

Figs. 50 and 51 are typical of metaphase in this hybrid.

Fig. 52.—Metaphase. One pair of chromosomes is lying transversely on the spindle.

Fig. 53.—Metaphase. The chromosomes are arranged more regularly than is usual. All the bivalents have retained the ring form.

Fig. 54.—Anaphase in polar view. Six chromosomes of each group of chromosomes lie in a ring whilst the seventh lies towards the centre.
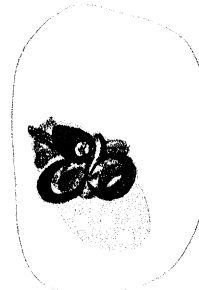
Mitotic Division.   (Figs. 55–56.)

Fig. 55.—Polar view of metaphase. There is apparently but little pairing between homologues.

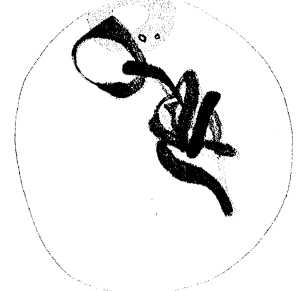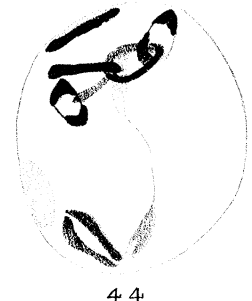Fig. 56.—Polar view of metaphase. Several of the chromosomes seem to be paired.
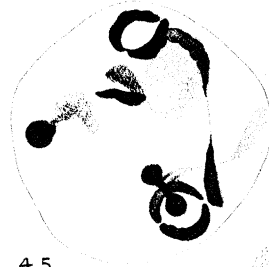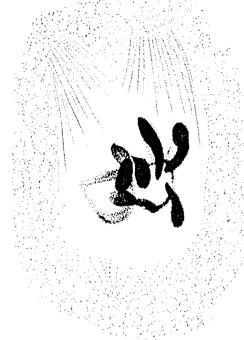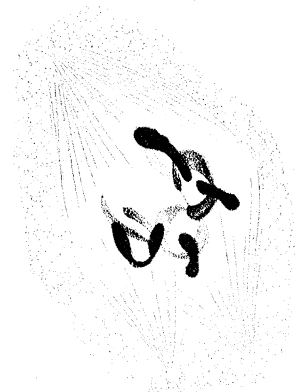
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F.M.L.S. del

Huth, London.

Oenothera ammophila × (biennis × rubricalyx).

Oe. ammophila × 33. 1.

1922.

1923.   3.(27 plants)

I. 1.   I. 2.   I. 7.   II. 1.   II. 5.

1924. 6. (6 A+4 B)*   7. (45 Bᴵ plants)   8. (69 Bᴵ plants)   9 (50 Bᴵ plants)   10. (2 A+2 B)
       I. 1.   I. 15.   I. 7.   I. 4.

I. 6.   III. 15.

1925. 2. (27 A+45 B)   3. (32 Bᴵ plants)   4. (38 Bᴵ plants)   6. (33 Bᴵ plants)   5. (25 Bᴵ plants)   7. (17 A+21 B)

I. 4. III. 3. V. 7. V. 12. I. 4. II. 1. II. 6. II. 10. II. 12. III. 1. III. 5. I. 5.   I. 8. I. 12. II. 4. III. 1. I. 2. I. 3. I. 6. I. 10. II. 2. II. 16. I. 6. II. 4. II. 10. II. 13. III. 2. L. 11. I. 15. II. 15.

1926.   8.   9.   10.   11.   12.   13.   14.   15.   16.   17. 21. 22. 23.   24. 18. 19.   20. 25. 26.
        (15 B)   (3 B) (4 Bᴵ) (1 Bᴵ) (1 Bᴵ) (5 Bᴵ) (3 Bᴵ) (11 A+28 B) (3 A) (3 A) (10 Bᴵ) (3Bᴵ) (20 Bᴵ) (5 Bᴵ) (22 Bᴵ) (20 Bᴵ) (9 Bᴵ) (11 A+7 B) (3B)
                                                                                       I. 9.   I. 6. II. 8.

I. 1. I. 3.   I. 3. III. 8.   II. 18×I. 3. II. 18×II. 9. II. 9×II. 7.

1927. 45   44.   46.   47.   5.   6.   7   8   9   10.   11.   48.   49.   14.   52.   53.   12   13.   50. 51.   54. 55.
      (4 B)   (10 A) (9 Bᴵ) (8 Bᴵ) (18 Bᴵ)(12 Bᴵ)(8A)(2 B)(6 A+6 B)(1 B)(3 A+8 B)(11 Bᴵ)(35 Bᴵ)(16 Bᴵ)(21 Bᴵ)(5 Bᴵ)(50 Bᴵ)(11 Bᴵ) 9 Bᴵ 15 Bᴵ (12 B)(7B)

* Three other plants were forming stems and could not be classified.

Fig. 2. Chart of Families.

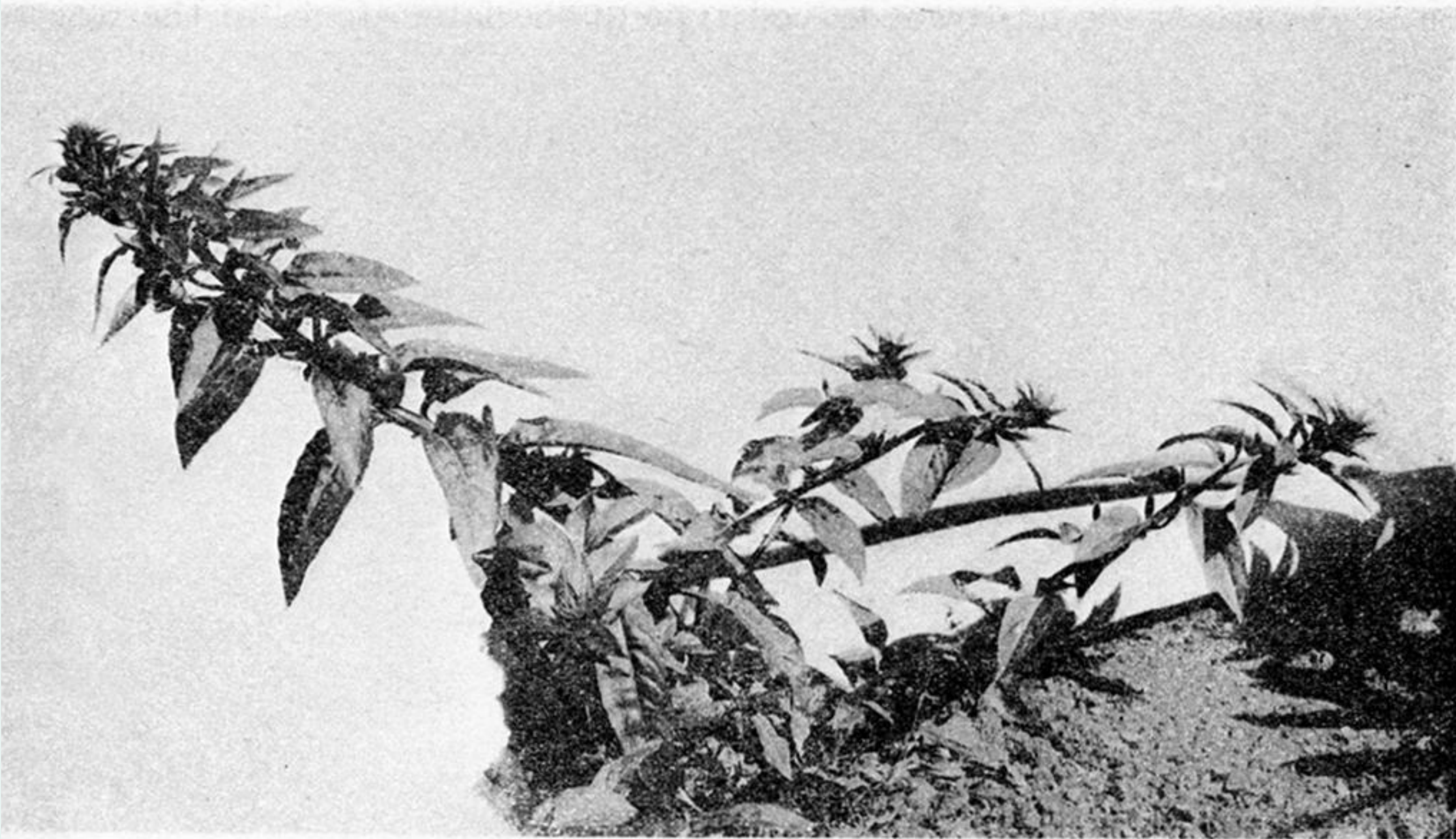(The cultures in circles were studied cytologically.)

Fig. 3.—*Oe.* (*biennis* × *rubricalyx*) × *ammophila*, F₁, showing oblique stems.

Fig. 4.—*Oe. ammophila* × (*biennis* × *rubricalyx*) F₁.

g. 5.—*Oe (biennis × rubricalyx) × ammophila*, F₂, Type A.

ig. 6.—*Oe. ammophila* × (*biennis* × *rubricalyx*),
F₂, Type B¹.

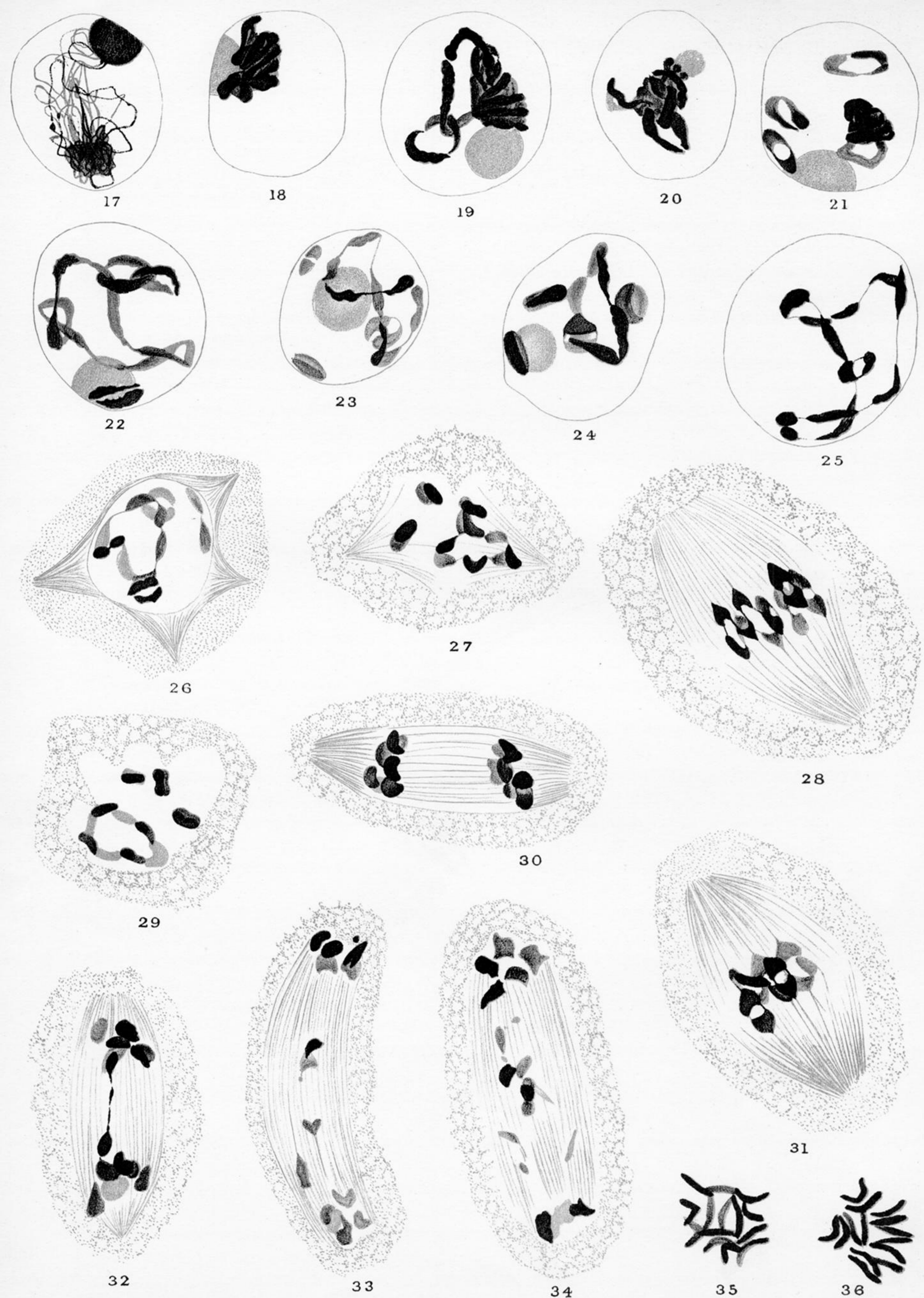g 7.—*Oe. ammophila* × (*biennis* × *rubri-calyx*), F_3, Type B.

Plate 89.

Oenothera ammophila × (*biennis* × *rubricalyx*).

Meiotic division. (Figs. 17–34.)

The cytological conditions in the broad-leaved and narrow-leaved types were precisely alike.

Fig. 17.—Narrow-leaved type. Open spireme. The pachynema is still very long and fine. Although the synizetic knot has not completely loosened, second contraction is imminent.

Fig. 18.—Broad-leaved type. Second contraction. The spireme has thickened considerably, and is again tightly entangled. It is connected to the pale-staining biconvex nucleolus.

Fig. 19.—Broad-leaved type. The second contraction knot has loosened slightly, and the thread can be seen to have become constricted into a number of parts. A single ring pair has been cut off from the spireme, but the two are interlocked.

Fig. 20.—Broad-leaved type. Two ring pairs of chromosomes have been segmented off from the spireme and are interlinked with it. A third loop, consisting of two chromosomes and projecting from the central knot, may give rise to a third bivalent.

Fig. 21.—Broad-leaved type. Three ring pairs of chromosomes have been set free from the second contraction knot.

Fig. 22.—Broad-leaved type. The second contraction knot has loosened, three ring pairs of chromosomes are now visible, and a large twisted ring of chromatin which has been constricted into eight parts. One bivalent is interlinked with the ring and with another pair.

Fig. 23.—Narrow-leaved type. Diakinesis. The nucleus contains a ring of eight chromosomes and three free ring pairs. The nucleolus, which is in bottom focus, and consequently appears spherical, has become very indistinct. The chromosomes are losing their spongy nature.

Fig. 24.—Broad-leaved type. Abnormal diakinesis. Six of the chromosomes are linked to form a ring, the remaining eight are paired.

Fig. 25.—Narrow-leaved type. Abnormal diakinesis. The nucleus is cut, and this section contains only thirteen chromosomes, eleven of which form a chain, while two form a small ring. The fourteenth chromosome is in the adjacent section, and occupies a position indicating that it was once in close proximity to the two overlapping ends of the chain.

Fig. 26.—Narrow-leaved type. Formation of the multipolar spindle. Chromosomes arranged in a contorted ring of eight with three free bivalents. The nucleolus has disappeared.

Fig. 27.—Narrow-leaved type. Multipolar spindle. The eight chromosomes of the ring have become slightly undulated, preparatory to assuming their appropriate positions on the bipolar spindle.

Fig. 28.—Narrow-leaved type. Metaphase. The spindle has become bipolar, and the chromosomes are arranged near the equatorial region. Adjacent chromosomes of the ring of eight have become attached to fibres from opposite poles.

Fig. 29.—Narrow-leaved type. Polar view of metaphase. The ring of chromosomes is undulated owing to the adjacent chromosomes being attached to fibres from opposite poles. The three bivalents lie towards one side of the spindle.

Fig. 30.—Broad-leaved type. Anaphase.

Fig. 31.—Broad-leaved type. Abnormal metaphase. The nucleus is cut, twelve chromosomes only being present. The chromosomes of the ring are not orientated in the usual way (see text).

Fig. 32.—Broad-leaved type. Abnormal anaphase. The connection between two of the chromosomes has so far failed to break away.

Fig. 33.—Broad-leaved type. Abnormal anaphase. Certain of the chromosomes are lagging.

Fig. 34.—Broad-leaved type. Abnormal anaphase. Five of the chromosomes have fragmented.

Mitotic Division. (Figs. 35–36.)

Fig. 35.—Metaphase in polar view. The chromosomes are scattered in the equatorial plate.

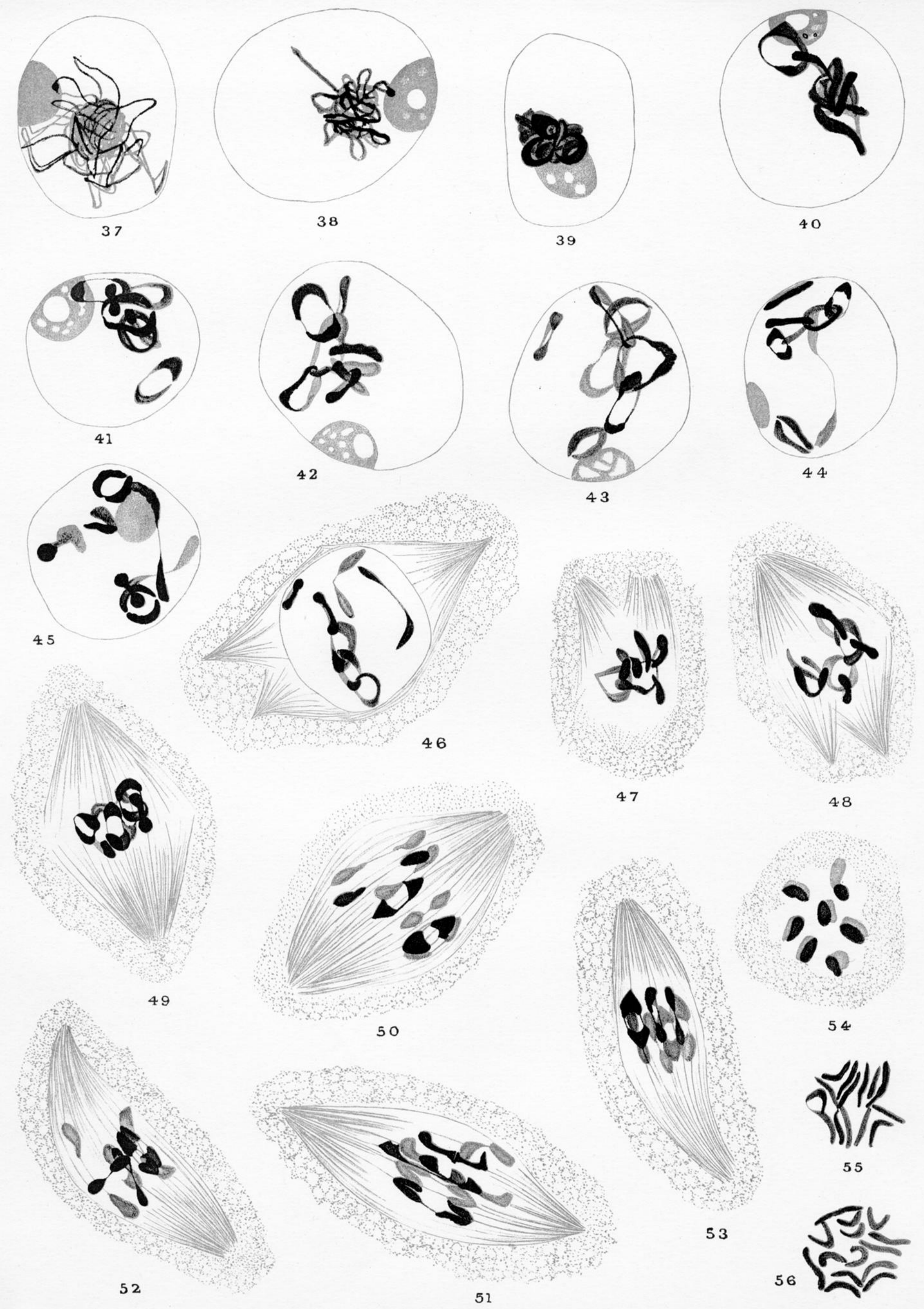Fig. 36.—Polar view of metaphase. The chromosomes are apparently mostly paired.

PLATE 90.

Oenothera (biennis × rubricalyx) × ammophila.

Meiotic Division. (Figs. 37–54.)

All figures are drawn from the narrow-leaved type, the broad-leaved not having been examined.

FIG. 37.—Open spireme. Numerous looped threads project from a central tangle. One of these loops is attached to the endonucleolus.

FIG. 38.—Beginning of second contraction. The pachytene thread has shortened and thickened, the loops being drawn into the central knot. One loop is attached to the endonucleolus which lies at the periphery of the vacuolate nucleolus.

FIG. 39.—The acme of second contraction. All the loops have now been drawn into the knot, which lies against the nucleolus. The spireme is considerably thickened.

FIG. 40.—The second contraction knot has loosened slightly. A single ring pair of chromosomes has been cut off but has remained interlinked with the spireme. The nucleolus is very vacuolate, some of the vacuoles containing crystalloid-like bodies.

FIG. 41.—The loosening of the second contraction knot. The pair of chromosomes which was cut off precociously has been set free, the other six bivalents, each of which forms a ring, are interlocked.

FIG. 42.—Diakinesis. Six of the ring pairs are interlocked whilst the seventh is free.

FIG. 43.—Diakinesis. Three of the ring pairs are free whilst the remainder are interlocked.

FIG. 44.—Diakinesis. In two cases the chromosomes constituting the bivalents are joined only at one end and consequently do not form rings. Three of the ring pairs are free whilst two are interlocked.

FIG. 45.—Diakinesis. Only three of the bivalents form rings, two of which are interlinked. In the case of the other bivalents the homologues lie end to end.

FIG. 46.—Formation of the multipolar spindle. Four of the ring pairs are interlinked to form a chain, whilst the fifth is free. The remaining bivalents do not form rings.

FIG. 47.—Multipolar spindle. Six of the ring pairs are still interlocked.

FIG. 48.—Multipolar spindle. Interlocking between the ring pairs still occurs.

FIG. 49.—Metaphase. Two ring pairs are still linked together. This condition is unusual at such a late stage. Two of the pairs lie obliquely on the spindle.

FIG. 50.—Metaphase. Some pairs are lying at the central region of the spindle whilst some homologues have already separated.

FIG. 51.—Metaphase. The chromosomes composing the bivalents which retained the ring form are now V-shaped. The other chromosomes now vary greatly in shape.

FIGS. 50 and 51 are typical of metaphase in this hybrid.

FIG. 52.—Metaphase. One pair of chromosomes is lying transversely on the spindle.

FIG. 53.—Metaphase. The chromosomes are arranged more regularly than is usual. All the bivalents have retained the ring form.

FIG. 54.—Anaphase in polar view. Six chromosomes of each group of chromosomes lie in a ring whilst the seventh lies towards the centre.

Mitotic Division. (Figs. 55–56.)

FIG. 55.—Polar view of metaphase. There is apparently but little pairing between homologues.

FIG. 56.—Polar view of metaphase. Several of the chromosomes seem to be paired.