Chromosome Organization in *Rosa*¹⁾

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Received February 17, 1931

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Introduction

Studies on the cytology of *Rosa* have heretofore been chiefly concerned with the chromosome numbers characteristic of the different species. They have been undertaken in the hope that they would throw light on the phylogeny and inter-relationship of the multitudinous forms. The existence of a polyploid series with the basic number seven, discovered by TÄCKHOLM (1922) has been confirmed by BLACKBURN and HARRISON, PENLAND, HURST, and myself.

1) Paper from the Department of Botany of the University of Michigan, No. 352, representing work carried on under a National Research Fellowship in the Biological Sciences.

The majority of the polyploid species is found in the two sections Cinnamomeae and Caninae. The greatest concentration of forms belonging to the Cinnamomeae is in Western Asia and in North America, while the Caninae are characteristic of Europe and eastern Asia. The Cinnamomeae resemble, in their chromosome constitutions and cytological behaviour, other large polyploid genera. The members of the Caninae, however, are unique among known organisms in having no more than seven bivalents at diakinesis, although the somatic numbers may be $4n$, $5n$, or $6n$, the remainder of the chromosomes remain unpaired. This unbalanced polyploid condition is maintained by facultative apomixis, and also by a special mechanism whereby the functional megaspore receives all the unpaired chromosomes, plus seven which have paired, while the functional pollen grain receives only seven chromosomes from the pairs (TÄCKHOLM 1922).

The cytological behaviour of the Caninae was interpreted by TÄCKHOLM to have been occasioned by ancient crossings between diploid and polyploid species, the chromosome constitutions of the F_1 from these becoming fixed and perpetuated by the process outlined above. Because a few species are hexaploid and show 7 bivalents and 28 univalent chromosomes at diakinesis, he assumed that one of their ancestors was a decaploid with $n=35$. HURST (1925, 1928) has proposed a classification of the genus *Rosa* based on the chromosome numbers, admitting this assumption of an ancestral decaploid form, which he has developed into a highly artificial system. He supposes that there are five distinct and unchanging chromosome sets in *Rosa*, which by purely static recombinations achieve the whole diversity of the genus. HURST's theory depends on two major assumptions (1) an unchanging condition of the chromosomes, and (2) the transmission of the sets as units in inheritance. These assumptions need not be rejected because they disagree with work on all other polyploid genera, for, as I have said, *Rosa* is unique in several important respects. I shall therefore examine HURST's two assumptions in the light of direct observation on the genus.

Material and Methods

During a recent visit to England I had the privilege of studying for a short time at the John Innes Horticultural Institution, and at the suggestion of Dr. C. D. DARLINGTON the following statistical study of chiasma-formation in *Rosa* was undertaken.

Anthers and root-tips of the dwarf polyantha rose "Orleans" were fixed in a medium strength FLEMMING's solution and stained by NEWTON's Gentian Violet method (see LACOUR 1931). Sections of root-tips were cut at a thickness of 10μ and anthers at 20μ , which gave a maximum number of uncut nuclei. Excellent preparations were obtained of pollen mother cells in which the points of contact between paired chromosomes could be seen to be effected by an exchange of partners between chromatids. Such points or nodes are referred to in this paper as chiasmata. Points of contact along the length of two chromosomes are called interstitial chiasmata, and those at the ends terminal chiasmata.

American material of *Rosa blanda* AIT. fixed in CARNOY's solution, and of *R. relict*a ERLANSON fixed in acetic alcohol, were also cut and stained in the same way as the rose "Orleans". It was possible to count the chiasma-frequencies, but this fixation was not completely satisfactory. The swelling effect of CARNOY's solution rendered the metaphase configurations obscure in some nuclei.

All drawings were made at bench level with a ZEISS camera lucida, with a ZEISS 1.5 mm. objective (N. A. 1.3), a ZEISS $30\times$ eyepiece and a tube length of 145 mm., to give a magnification of 6200. For Fig. 22 the tube length was increased and a magnification of approximately 9000 was obtained. Figs. 2 and 5 were drawn by Dr. DARLINGTON, with a tube length of 195 mm. which gave a magnification of 9600; they have been reduced to two-thirds in reproduction. Figs. 26 and 27 were drawn with a $20\times$ eyepiece at a magnification of 4500. For the sake of clearness, unconnected groups of chromosomes in nuclei at diakinesis and metaphase have been drawn separately.

Fig. 1. Somatic metaphase from root tip of the rose "Orleans".

Somatic Chromosomes

The rose "Orleans" is a diploid with fourteen somatic chromosomes. Fig. 1 shows a somatic metaphase plate from a root tip. The chromosomes vary from 1.5μ to nearly 3μ in length and have both primary and secondary constrictions. The attachment constrictions are approximately median in half of them and subterminal in the rest.

There are evidently not seven pairs of identical chromosomes, and a trabant was seen on only one.

Analysis of Chiasmata

(1) *The chiasma theory of chromosome pairing.*

Until recently our understanding of the conditions governing meiotic pairing was vague. The careful studies of NEWTON and DARLINGTON on *Tulipa*, *Hyacinthus*, *Fritillaria* and the *Tradescantiae* (NEWTON 1927, NEWTON and DARLINGTON 1929, 1930, DARLINGTON 1929-1931) have given a consistent and logical explanation of chromosome pairing applicable equally in animals and plants. They have substantially demonstrated that, at pachytene, association is constantly parasyntaptic, that the attraction of every particle of a chromosome is specific and that homologous parts associate side by side in pairs.

DARLINGTON's theory of pairing rests on his observations (1) that "pairing at metaphase depends on the formation of a chiasma between the chromosomes that have paired at pachytene" (DARLINGTON 1930) and (2) "that the occasion of pairing was simply the random formation of chiasmata" (DARLINGTON 1929 b), or the exchanges of partners among chromatids in a four strand system at diplotene. This view was rendered possible by the interpretation of end to end association as due to a terminal chiasma, an interpretation that has been statistically verified (DARLINGTON 1931 a). In the present study this interpretation has been followed.

A widespread characteristic of meiosis is the repulsion apparently exercised towards each other by paired chromosomes. This repulsion may be localized in the attachment constriction. It is seen to reach a maximum at diakinesis, when the chromosomes are undergoing rapid linear contraction; and related to these two phenomena is a diminution in the number of chiasmata. DARLINGTON attributes the disappearance of some of the chiasmata between diplotene and metaphase to a terminalization of chiasmata caused by the falling apart of the homologous chromosomes; some of the interstitial chiasmata slip along the chromatids and become terminal (DARLINGTON 1931 c). His studies on *Primula sinensis* (DARLINGTON 1931 b) have demonstrated the origin of multiple chiasmata through terminalization. SAX (1930) attributes the loss of some interstitial chiasmata to a rupture of the crossed chromatids.

(2) *Chiasmata in a diploid garden rose.*

The dual nature of the chromosomes at meiosis in the pollen mother cells of *Rosa* could only be seen at the chiasma points where the chromatids usually become opened out or pulled apart. Almost all the points of contact between paired chromosomes were unmistakably chiasmata, and, as in *Lilium* (BELLING 1928 b), and in *Secale* (SAX 1930), there is practically no twisting of the associated chromosomes about each other from diplotene onwards.

Fig. 2. Chromosome pairs at diakinesis in the rose "Orleans", showing interstitial and terminal chiasmata. The number of chiasmata in each pair is indicated by a numeral. (Drawn by C. D. DARLINGTON).

The main types of chiasmata observed in bivalents in pollen mother cells of the rose "Orleans" at diakinesis, are shown in Fig. 2. Individual bivalents have been selected and drawn separately, and the number of chiasmata in each pair is given by a numeral. The paired chromosomes may form a ring with two chiasmata, one of which is usually terminal and the other either a terminal or a sub-terminal interstitial chiasma. One terminal and one interstitial chiasma give a Y-shaped bivalent. Configurations in the form of a cross occasionally possess two interstitial chiasmata close together in the central region of the chromosomes; but they more frequently have only one median interstitial chiasma; and in that case the chromatids are

often pulled apart at the chiasma point and all four stands can be clearly seen (Fig. 2). Pairs united by one chiasma may have the node at any point along the chromatids: or the association may be strictly terminal, in which case the ends of the two paired chromosomes are frequently not actually in contact with one another but are separated by a clear space in which a connecting thread, or pair of threads, can often be discerned. When there is a sub-terminal interstitial chiasma the sister-chromatids are sometimes separated from each other for some distance along each chromosome, as shown also in *Secale* by SAX (1930).

Fig. 3. Late diplotene in the rose "Orleans", with five bivalents and one quadrivalent. Total number of chiasmata 19.

Fig. 4. Three bivalents of late diplotene with high chiasma-frequencies in the rose "Orleans"

The frequencies of terminal and interstitial chiasmata at late diplotene, early and late diakinesis and at metaphase, were counted. The total number of chiasmata in a given number of whole nuclei is shown for each of these four stages of meiosis in Table 1. The interstitial chiasmata were counted separately, and the proportion of these occurring at each stage is given in column six of the table. It is plain that the number of interstitial chiasmata falls off rapidly; they are more than three times as frequent at late diplotene as at metaphase. This together with the regular reduction in the mean number of chiasmata

Table 1

Analysis of chiasmata in *Rosa* hyb. hort. "Orleans", demonstrating terminalization.

	No. of divisions	No. of pairs of chromosomes*	Total No. of Xta	No. of Xta interstitial	Mean No. of Xta per bivalent	Proportion of Xta interstitial	Highest No. of Xta observed in any bivalent
Late Diplotene (fig. 3)	3	21	56	43	2.66	.77	5 (fig. 4)
Early Diakinesis (fig. 5)	5	35	63	47	1.80	.75	4
Late Diakinesis (fig. 7)	11	77	127	68	1.65	.53	3
Metaphase (fig. 10)	10	70	107	26	1.53	.24	2

* Or their equivalent in unpaired chromosomes and quadrivalents.

per bivalent, from 2.66 at late diplotene to 1.53 at metaphase, clearly demonstrates the effects of terminalization. Figs. 3, 5, 7 and 10 are examples of four successive stages in meiosis which were taken for analysis of chiasma-frequency. Very few whole nuclei were found

at the late diplotene stage (see Fig. 3). The highest number of chiasmata seen in any bivalent at this stage was five in a cut nucleus. Three bivalents at late diplotene with high numbers of chiasmata have been drawn separately in Fig. 4. In Fig. 10 the seven pairs at metaphase have been drawn separately for clarity. This is possible because of the transparency of the gentian violet stain. If Fig. 10 and other metaphase figures (Figs. 11-13) are compared with the figures of earlier stages in meiosis, the principles of chiasma-formation which govern the configurations are made clear. This analysis demonstrates the truth of the

Fig. 5. Early diakinesis in the rose "Orleans" with seven bivalents. The number of chiasmata in each bivalent is given. (Drawn by C. D. DARLINGTON).

Fig. 6. Early diakinesis in the rose "Orleans" with five bivalents and a quadrivalent. Total number of chiasmata 17.

Fig. 7. Late diakinesis in the rose "Orleans" with seven bivalents. Total number of chiasmata 11.

Fig. 8. Late diakinesis in the rose "Orleans" with five bivalents and one quadrivalent. Total number of chiasmata 11.

Fig. 9. Late diakinesis in the rose "Orleans" with 2 univalents, 4 bivalents and a quadrivalent. Total number of chiasmata 11.

statement of NEWTON and DARLINGTON (1930) that "in plants and animals with smaller chromosomes where the existence of chiasmata has usually been ignored, the superficial appearance is merely the result of extreme condensation of the loops formed at diplotene."

Figs. 10-13. Metaphase complements from four whole nuclei in the rose "Orleans." Chiasmata totals are 11, 10, 8 and 12 respectively. Note two univalents in Fig. 12 and the quadrivalent in Fig. 13 with 2 terminal and 2 interstitial chiasmata.

Table 2 shows the frequencies of chiasmata (from 1-4) in bivalents of uncut nuclei at the same four stages of meiosis dealt with in Table 1. A small proportion of nuclei in this rose showed a complete failure of chiasma-formation in one pair of chromosomes, and a still smaller proportion contained quadrivalent configurations. Where one has quadrivalents (e.g. Fig. 9) it is impossible, for statistical purposes, to consider the number of chiasmata per bivalent. One can only say what the chiasma frequency is per half bivalent; so that in Fig. 9, I say that the four half bivalents present in the quadrivalent have chiasmata frequencies of 1, 2, 2, and 1. In Table 2 chiasma-frequency has been expressed as a percentage which is given beside the actual number of bivalents. Fig. 14 shows the data contained in Table 2 expressed

Fig. 14. Frequency polygons of number of chiasmata in bivalents (or their equivalents in unpaired chromosomes and quadrivalents) in the rose "Orleans" at four stages of meiosis. *L. Dip.*, late diplotene; *E. Dk.*, Early diakinesis; *L. Dk.*, late diakinesis; *M*, metaphase.

Table 2

Chiasma-frequency in the rose "Orleans".

Nos. of chiasmata	0	1	2	3	4	Total No. of pairs*
Nos. of pairs at Late Diplotene	0 0%	1 4.8%	7 33.3%	11 52.4%	2 9.5%	21
Early Diakinesis	1 2.9%	11 31.4%	16 45.7%	6 17.1%	1 2.6%	35
Late Diakinesis	2 2.6%	25.5 33.12%	47. 61.04%	2.5 3.24%	0 0%	77
Metaphase	2 2.9%	38 54.3%	30 42.8%	0 0%		70

* Or their equivalent in unpaired chromosomes and quadrivalents.

graphically in four curves. These demonstrate diagrammatically the effects of terminalization. The chiasma frequency at late diplotene falls on a normal frequency curve with the mean at 2.66, demonstrating the random formation of chiasmata at this stage. The curves for early and late diakinesis are very similar to one another, but in the latter there are no bivalents with four chiasmata.

(3) *Chiasmata in a wild diploid rose.*

Some material of *Rosa blanda* was analysed for chiasma-formation in the pollen mother cells, by the same method as that used for the rose "Orleans." No pollen mother cells were available at late diplotene stage; however, Table 3 gives the analysis of chiasmata at early and late diakinesis and at metaphase. Table 4 contains the chiasma fre-

Table 3

Analysis of chiasmata in *Rosa blanda* (7N29).

	No. of divisions	No. of potential pairs of chros.	Total No. of Xta	No. of Xta interstitial	Mean No. of Xta per bivalent	Proportion of Xta interstitial	Highest No. Xta in any bivalent
Early Diak.	11	77	149.5	98	1.94	.65	3
Late Diak.	11	77	113.5	56	1.47	.50	3
Metaphase	18	126	166	52	1.31	.31	2

Table 4

Chiasma-frequency in *Rosa blanda*, 7N29.

No. of chiasmata	0	1	2	3	Total No. of pairs*
Early Diakinesis	.5 0.65%	17.5 22.72%	45 58.44%	14 18.2%	77
Late Diakinesis	1 1.3%	40.5 52.6%	33.5 43.5%	2 2.6%	77
Metaphase	2 1.6%	82 65.1%	42 33.3%	0 0%	126

* Or their equivalents in unpaired chromosomes, trivalents and quadrivalents.

quencies at these stages, and the same data are expressed graphically in Fig. 15. If Tables 1 and 3 and Figs. 14 and 15 be compared, a striking similarity is apparent between the manner of chromosome pairing in these two unrelated diploid roses. The rose "Orleans" belongs to the *Synstylae* section of the genus and is closely related to the Chinese species *R. multiflora* THUNB; it was placed on the market by LAVAVAS-

Fig. 15. Frequency polygons of number of chiasmata in bivalents (or their equivalents in unpaired chromosomes or multivalents) in *Rosa blanda* Ait. 7N29, at three stages of meiosis. *E. Dk.*, early diakinesis; *L. Dk.*, late diakinesis; *M*, metaphase.

SEUR in 1909. *R. blanda* belongs to the section *Cinnamomeae*. The buds which were used in this study were taken from a plant which was grown from seed of a wild individual in Mackinaw County, Michigan. It is number 7N29 in my cultures at the Botanical Garden of the University of Michigan.

The mean number of chiasmata at diakinesis is 1.72 per bivalent in the rose "Orleans" and 1.75 per bivalent in *Rosa blanda* 7N29, when the data for early and late diakinesis are lumped together. A com-

parison of Figs. 14 and 15 shows that terminalization is slightly more complete in the *Rosa blanda* plant. These data show that we are here dealing with the same phenomena in the two cases.

(4) *Chiasmata in other genera.*

The first statistical studies of chiasmata in flowering plants were made by BELLING in *Hyacinthus* and in *Lilium* (BELLING 1927a, 1927b, 1928 b). He failed to recognize the significance of terminal association between paired chromosomes, and therefore, as DARLINGTON has pointed out (DARLINGTON 1931 a), the reduction in the total number of chiasmata between early and late diakinesis appears greater in his tables than it actually is. DARLINGTON (1929 b) analysed the chiasmata in aneuploid *Hyacinthus* and showed that the chiasma number varies with the length of the chromosome. He found at metaphase 3-4 chiasmata in the long type of chromosome and rarely more than one in the short type, a consequence of the random formation of chiasmata at diplotene.

Chiasma-frequency has been measured in few dicotyledons, because they provide less favorable material for such studies than the monocotyledons with their larger chromosomes. Where this has been attempted, as in *Lathyrus* and *Vicia* (MAEDA 1930 a, 1930 b), *Primula* (DARLINGTON 1931 b) and *Matthiola* (PHILP & HUSKINS 1931), observed conditions completely fulfill the requirements of DARLINGTON's theory: pairing is parasynaptic and chiasmata are formed at random up to five per bivalent. The chiasma-frequencies observed by PHILP & HUSKINS in *Matthiola incana* are similar to those found in the rose "Orleans" and in *Rosa blanda*. The mean total chiasma-frequency per bivalent in *Matthiola* is 2.26 at pre-diakinesis stages and 1.54 at metaphase.

Structural Hybridity

(1) *Chromosome configurations in diploids.*

In the rose "Orleans," among forty whole nuclei, at diakinesis, twenty-nine showed seven bivalents (Figs. 5 and 7). In seven others one pair of chromosomes had failed to form chiasmata, giving six bivalents and two univalents. In three nuclei five bivalents and a quadrivalent were found (Figs. 6, and 8). One nucleus among the forty showed four bivalents, a quadrivalent and two univalents (Fig. 9). The frequencies of these configurations at diakinesis and at metaphase are given in Table 5. The proportion of potential biva

lents with no chiasmata is 2.5% at diakinesis, and 2.9% at metaphase. The proportion of chromosomes involved in quadrivalents is 2.9% at both stages. There is therefore no significant difference in the association between the stages. This is a statistical proof that the end to end association at metaphase is functionally equivalent to the interstitial chiasma of prophase. The end to end association is a terminal chiasma.

Table 5

Chromosome configurations at diakinesis and metaphase in whole nuclei in the rose "Orleans"

	6II + 2I	4II + 1IV + 2I	5II + 1VI	7II	Total nuclei	Total potential pairs	Proportion 0 Xta	Proportion of chromosomes in quadrivalents
Diakinesis	7	1	3	29	40	280	2.5%	2.9%
Metaphase	2	0	1	7	10	70	2.9%	2.9%

The association of more than two chromosomes in a diploid has been observed in several genera: *Enothera*, *Tradescantia*, *Rhoeo*, *Datura*, *Pisum*, *Campanula*, *Zea*, *Matthiola*, (PHILP & HUSKINS 1931), *Briza* and *Anthoxanthum* (KATTERMANN 1930). If "the side by side conjugation of homologous parts of chromosomes is a universal condition of meiosis" (DARLINGTON 1929 a), then rings of four or more chromosomes in a diploid must be due to segmental interchange between non-homologous chromosomes, as suggested by BELLING & BLAKESLEE (1924, 1926, BELLING 1927 c) with respect to *Datura*, and corroborated by DARLINGTON (1929 c, 1929 a) for *Tradescantia*, *Enothera* and *Campanula* (GAIRDNER & DARLINGTON 1930). DARLINGTON's prediction (DARLINGTON 1929 c) that rings of four, formed from the same cause, would be discovered in diploids of widely different genera, has been amply fulfilled.

The occurrence of rings of four in the rose "Orleans" therefore, shows that it is a *structural hybrid* as well as a hybrid in the general sense. The low frequency of quadrivalent formation in this plant is due to the smallness of the exchanged segments, since chiasma-formation is fortuitous and random (GAIRDNER and DARLINGTON 1930). Chiasma-frequency at diplotene is only 2.66 per entire bivalent, and there is no reason to suppose that the exchanged fragments are even

Figs. 16-21. Nuclei at diakinesis in *Rosa blanda* 7N29, showing multivalent configurations (Figs. 16, 17, 18, 20 and 21) and interlocking pairs (figs. 19 and 21). The arrows indicate that a chromosome group has been moved in drawing.

a quarter of the length of a whole chromosome. In *Campanula* GAIRDNER and DARLINGTON (1930) found complete terminalization which gave rings or chains of four at metaphase. In the rose "Orleans" terminalization is partially arrested and some interstitial chiasmata persist in the quadrivalent groups at metaphase (Fig. 13).

Fig. 22. Quadrivalent configurations at diakinesis in *Rosa blanda* 7N29 interpreted diagrammatically. 2' has a triple chiasma, it has 3 homologous segments, i.e. reduplication, chromosomes involved: *ec, ed, df, df*. 1', 3', and 4' can be explained on a basis of segmental interchange, chromosomes involved: *abc, abd, ec, ed*. In 1' and 4' the interchanged segment is small and has arrested terminalization.

A ring of four has also been seen in two wild diploid plants of *Rosa blanda* from Michigan, which have been raised from seed. The individual 7N29 has bivalent, trivalent, quadrivalent and sexivalent configurations at diakinesis (see Figs. 16-21). At this stage, 5.45% of the chromosomes are involved in multivalent configurations; multivalent groups are also present at metaphase (see Figs. 24 and 25). Table 6 shows the frequencies of the different types of configurations seen in seventy-four whole nuclei at diakinesis and in thirty-three whole nuclei at metaphase. At diakinesis three groups of

Figs. 23-25. Metaphase complements in *R. blanda* 7N29. Fig. 23, 7 bivalents and 2 univalents, 9 chiasmata. Fig. 24, 4 bivalents and 2 trivalents, 9.5 chiasmata. Fig. 25, 4 bivalents and 1 sexivalent, 9.5 chiasmata.

Table 6

Chromosome configurations at diakinesis and metaphase in whole nuclei
of *Rosa blanda* 7N29.

	6II + 2I	4II + 1IV + 2I	5II + 1III + 1I	5II + 1IV	4II + 1VI	4II + 2III	7II	Total nuclei	Total poten- tial pairs	Propor- tion 0 Xta	Proportion of chromo- somes in multi- valents
Diakinesis	1	1	2	10	1		59	74	518	0.58%	5.45%
Metaphase	2			4	1	1	25	33	231	0.85%	6.06%

two bivalents were found in which the pairs were not connected by chiasmata but had looped around each other at diplotene (Fig. 19). Such linked or interlocked bivalents, not connected synaptically, have been observed in *Datura*, as shown in BLAKESLEE's diagram (BLAKESLEE 1929, Table 1). DARLINGTON (1929 b) found a similar condition in tetraploid *Hyacinthus*, and refers to this occurrence also in certain Orthoptera.

In Fig. 21, a pair of chromosomes united at both ends by a terminal chiasma are looped through another pair of chromosomes which are themselves part of a quadrivalent group. A true sexivalent configuration, in which all six members are connected by chiasmata, is shown in Fig. 20. The existence of interlooped configurations makes difficult the interpretation of some multivalent groups at metaphase; nevertheless the proportion of multivalent groups at this stage closely approximates the proportion at diakinesis, as shown in Table 6.

(2) Chromosome structure.

In Fig. 22, four quadrivalents configurations seen in the individual 7N29 have been drawn separately at a magnification of approximately 9000. The quadrivalent 1' is the same as that shown in the whole nucleus in Fig. 18. In Fig. 22, the configuration 2' has a triple chiasma. It therefore has three homologous segments; if we accept pairing as "the final criterion of the homology of chromosomes" (DARLINGTON 1929 b); and can be explained on a basis of reduplication. The four chromosomes involved may be designated as *ec*, *ed*, *df* and *df*. The configuration 3' can be explained on a basis of segmental interchange between non-homologous chromosomes, as can also 1' and 4'. In these three quadrivalent groups, the chromosomes involved may be desig-

nated as *abc*, *abd*, *ec* and *ed*. In the configurations 1' and 4' terminalization has apparently been arrested by the small segment *d*; an interpretation suggested by DARLINGTON (1929 c) and adopted in lettering these figures.

It becomes increasingly clear that the pairing of chromosomes is not a straight-forward indication of their differentiation, as used to be assumed by workers with *Triticum*, *Nicotiana* and *Rosa*, but must be considered "in relation to chromosome length, chiasma-formation and the conditions of survival in a polyploid" (DARLINGTON 1930). HURST's assumption of an unchanging condition of the chromosomes in *Rosa* is refuted by the evidence of segmental interchange in diploids.

(3) Pollen sterility.

The individual rose "Orleans" used in this study showed 45% of its pollen grains without contents at maturity. The *Rosa blanda* plant 7N29 had 43.4% of bad pollen in 1930. High pollen sterility has been shown to occur among wild diploid and polyploid rose species with a balanced chromosome complement (ERLANSON 1931). A pollen sterility of about 50% has been found associated with structural hybridity due to segmental interchange in *Datura* (BLAKESLEE 1929), in *Pisum* (RICHARDSON 1929) and in *Zea* (BURNHAM 1930). HÅKANSSON (1929) did not give the fertility of the race of *Pisum* in which he found a ring of four in a diploid, but BURNHAM (1930) shows it to be about 50% in the line with abnormal linkage.

Figs. 26-27. Late first anaphase in *R. blanda* 7N29. In Fig. 26 one univalent has divided at first metaphase. Fig. 27 two univalents lagging.

Figures 26 and 27 show two pollen mother cell nuclei of the plant 7N29 at the end of first anaphase. In Fig. 26, a lagging univalent has divided giving 8 bodies at one pole and 7 at the other; in Fig. 27 two univalents are lagging. Such chromosome behaviour in addition to structural hybridity will augment sterility.

(4) *Chromosome configurations in a tetraploid rose.*

A preparation of the anthers of *Rosa relict*a was found favorable for the study of chiasma-formation at diakinesis in a tetraploid. Rings of four chromosomes have been observed in several tetraploid rose species (ERLANSON 1928), but these are doubtless homologous elements brought together by polyploidy. Only rings of five or more in a tetraploid can be evidence of segmental interchange (DARLINGTON 1929 a).

*Rosa relict*a shows quadrivalent configurations of chromosomes in a majority of the cells at diakinesis. An occasional cell has fourteen distinct pairs (Fig. 29); chiasma-formation has here failed between two of the four homologs present. Multivalent configurations in which five, six or eight chromosomes are connected to one another by chiasmata are frequent. Five nuclei at diakinesis are shown in Figs. 29-33.

Chiasma-frequency in *R. relict*a is similar to that found in the diploid individuals. The mean number of chiasmata per potential bi-

Table 7

Analysis of Chiasmata in *Rosa relict*a at Diakinesis.

No. of Divisions	No. of Potential pairs of chromosomes	Total No. of Xta	No. of Xta interstitial	Mean No. of Xta per bivalent	Proportion of Xta interstitial	Highest No. of Xta in any bivalent
11	154	264	110.5	1.71	0.42	4

Table 8

Chiasma-frequency at diakinesis in *Rosa relict*a.

No. of chiasmata	0	1	2	3	4	Total No. of pairs*
	1.5 1%	56 36.4%	82.5 53.6%	13 8.4%	1 0.6%	154

* Or their equivalents.

valent in ten whole nuclei at diakinesis was 1.71, and the maximum number observed in one bivalent was four. The data are given in Table 7. Table 8 shows the frequencies of 0-4 chiasmata among potential bivalents at diakinesis. The numbers of chromosomes found as univalents and of those involved in the various synaptic configurations

Fig. 28. Frequency polygon showing distribution of 308 chromosomes in various configurations in the tetraploid *R. relictata* at diakinesis.

Table 9

Types of chromosome configurations in eleven whole nuclei at diakinesis, in *Rosa relictata*. Total number of chromosomes = 308.

Configuration	I	II	III	IV	V	VI	VII	VIII
No. of Chros.	3	190	9	72	5	6	7	16
%	1%	62%	3%	23%	2%	2%	2%	5%

in eleven whole nuclei are given in Table 9. These data have also been expressed as percentages and are shown graphically in Fig. 28.

29

Fig. 29. *R. relict*a, diakinesis, 14 bivalents. Total chiasmata 23.

30

Fig. 30. *R. relict*a, diakinesis, 1V+1IV+1III+8II. Total chiasmata 21.

*Rosa relict*a is an extremely dwarf tetraploid from northern Illinois. It has highly sterile pollen, the amount of empty grains varying from 56-88%, (ERLANSON 1931).

The present cytological study has shown that it is a structural hybrid. It is evident that the members of the chromosome sets undergo differentiation independently after they are brought together in polyploid roses, as has been found to be the case in polyploids in the genera *Tradescantia* and *Prunus* (DARLINGTON 1929 c, 1930), and in *Aucuba* (MEURMAN 1929). Both of HURST's assumptions therefore become untenable. The poly-

Fig. 31. *R. relict*a, diakinesis, 1VIII+2IV+1III+4II+1I. Total chiasmata 26.5.

morphy for which the large polyploid genera are notorious now becomes understandable from direct cytological observations. Structural

Fig. 32. *R. relictata*, diakinesis, $2_{IV} + 1_{III} + 8_{II} + 1_{I}$.
Total chiasmata 28.

Fig. 33. *R. relictata*, diakinesis, $1_{VI} + 1_{IV} + 9_{II}$.
Total chiasmata 22.5.

change has shown that not only do chromosome-sets not behave as units in inheritance (and evolution) but chromosomes themselves are complexes of independently heritable fractions.

(5) *The Hypothesis of Segmental Interchange.*

Segmental interchange between non-homologous chromosomes was first called upon by BELLING & BLAKESLEE (1924 et seq.) to account for the chains of three and five, and other abnormal configurations, found in secondary trisomic *Datura* hybrids. It was also suggested that segmental interchange might be responsible for BELLING's earlier findings of semi-sterility in species crosses of *Mucuna* (*Stizolobium*) (BELLING 1925). In these first papers (BELLING & BLAKESLEE 1924, 1926), and in the next article (BELLING 1927c), BELLING was dealing chiefly with trivalent configurations in secondary trisomic *Datura* mutants. On the assumption of segmental

interchange in the "B race" of *Datura*, he expected quadrivalent configurations (rings of four) in the F_1 diploid hybrids between the B race and other lines; this expectation was fulfilled. He suggested tentatively (BELLING 1927 c) that this might be the mode of origin of similar configurations in *Enothera*. At that time, and even now in some instances, cytologists did not admit the universality of the parasynaptic mode of chromatid pairing. BELLING (1927) speaks of "2n different kinds of attraction" between the chromosomes in a diploid *Datura*, showing that he was not considering the attraction between the ultimate particles of the individual chromosomes, which NEWTON & DARLINGTON (1929) have shown are the real units when homology is to be considered.

HÅKANSSON (1928) applied the theory of segmental interchange to the *Enothera* data, but was unable to account for the half mutants. The only satisfactory and logical application of this mechanism to *Enothera* has been that given by DARLINGTON (1929 a), who combines the hypothesis of segmental interchange with the hypothesis of parasynapsis and pairing by chiasma-formation, and brings this heretofore anomalous genus into line with other organisms with respect to its cytological behaviour.

In spite of the comprehensive studies which have been made of the cytology of *Datura*, the chromosome configurations have been illustrated only at metaphase, after terminalization has occurred, and no picture of the chiasmata can be obtained from the accounts of BELLING & BLAKESLEE. BELLING (1928 a) reported that nodes may be seen at diplophase and early diaphase in *Datura*. An examination of the earlier stages of meiosis would, I believe, show a greater complexity of structural variation in *Datura*, where the "humps" referred to by BLAKESLEE & BERGNER (1930) are perhaps persistent interstitial chiasmata of the kind illustrated in the ring of four in *R. blanda* (Fig. 22, 1'). Here terminalization has probably been arrested by a change of homology as explained above.

Conditions in Polyploid Roses

This study of the chromosome organization in *Rosa* demonstrates a condition of complexity in which not chromosomes as wholes, but ultimate constituent parts, must be considered in order to understand the homologies involved and the variations that have produced them.

DARLINGTON (1928) pointed out that genetical behaviour of cereals showed the independent pairing of members of the same set of chromosomes in a hybrid; some pairing by autosyndesis, some by allosyndesis. The same is true of the pairing of chromosomes, as shown by the segregation, in a tetraploid *Rubus* hybrid (CRANE & DARLINGTON 1928). These results should be applicable to *Rosa*, for in this genus there is no more cytological evidence for chromosome sets behaving as units than in any other.

Recent cytological research has shown that the chromosomal homologies among polyploids are highly complex. Chromosomes which have descended from originally homologous structures, even though they are so far altered as to be unable to undergo synaptic pairing, may sometimes reveal their affinity by arranging themselves in definite groups at first metaphase and later. This has been called "secondary pairing" by DARLINGTON (1928). An examination of my earlier figures (ERLANSON 1928) shows that it occurs among hexaploid and octoploid roses.

Two hexaploid rose species which possessed only one set of seven homologous chromosomes in common, if crossed, would give rise to an unbalanced hexaploid with seven paired and thirty-five unpaired chromosomes,—of the type of *R. Jundzillii* BESS. in the section Caninae. The same result would be obtained from an octoploid crossed with a tetraploid, if they had only one similar set of homologous chromosomes each. An unbalanced pentaploid of the type of *R. rubiginosa* SM. might arise from a hexaploid crossed with a tetraploid, as well as from an octoploid and a diploid, as suggested by TÄCKHOLM. It is therefore unnecessary to assume the existence of a decaploid ancestral rose species in post-pleistocene times to account for the unbalanced hexaploids in the Caninae, since the origin of the unbalanced polyploids is not limited (as TÄCKHOLM thought) to hybridization between polyploid and diploid species, but might result from a cross between two polyploids.

Summary

1. The object of this study was to discover whether the chromosome behaviour in *Rosa* was in agreement with the chiasma theory of chromosome pairing.

2. Somatic chromosomes in *Rosa* showed primary and secondary constrictions and varied in length from about 1.5μ to 3μ . Pairing at diakinesis was found to be by chiasmata.

3. Chiasma-frequency was analysed in pollen mother cells of two diploid roses, a) the polyantha pompom garden rose "Orleans," and b) a wild plant of *Rosa blanda*. The mean number of chiasmata was of the same order in both individuals, namely 1.72 and 1.75 per bivalent at diakinesis, and 1.53 and 1.31 per bivalent at metaphase. The effect of terminalization was apparent in the regular falling off of the mean number of chiasmata per potential bivalent, and in the reduction in the proportion of interstitial to terminal chiasmata from early diakinesis to metaphase. A small proportion of chromosomes failed to form chiasmata and therefore appeared as univalent chromosomes.

4. Quadrivalent configurations were found at diakinesis and at metaphase in both the diploid roses: a phenomenon correlated with structural hybridity of the chromosomes. The fact that a similar proportion of the chromosomes were present as univalents, and involved in quadrivalents, at metaphase as at diakinesis, is offered as a proof that end to end associations are terminal chiasmata.

5. *Rosa blanda*, showed a few sexivalents as well as bivalent, trivalent and quadrivalent configurations at diakinesis. The structure of the chromosomes in some of the quadrivalents has been interpreted on the basis of segmental interchange in conjunction with parasynapsis. The plant is shown to be $3n$ for one short segment.

6. The diploid individuals both showed approximately 50% of bad pollen.

7. The highly sterile tetraploid *Rosa relict*a was found to have a mean chiasma-frequency of 1.71 per potential bivalent at diakinesis. Quadrivalents were frequent and the presence of multivalents involving 5, 6, and 8 chromosomes showed that the plant was also a structural hybrid.

8. Pairing in *Rosa* is by chiasmata as in other organisms. Failure of pairing as well as multivalent pairing involving segmental interchange affect part of the chromosome complement and not the rest. HURST's theory of seven differential septets of chromosomes in *Rosa* is thus proved untenable by direct cytological observation.

9. The phenomenon of "secondary pairing" is exhibited by some polyploid roses.

10. An alternative hypothesis is given to account for the unbalanced polyploid species of the Caninae, which does not involve a hypothetical decaploid ancestral form.

I wish to acknowledge my indebtedness to the Director of the John Innes Horticultural Institution for the use of laboratory facilities. I am also grateful to Dr. C. D. DARLINGTON for helpful suggestions and advice.

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