Secondary Pairing in *Brassica oleracea*

By

D. G. Catcheside D. Sc.

Dept. of Botany, University of London, King's College

Previous studies (Catcheside, 1934; Alam, 1935) have suggested that *Brassica* species are secondary polyploids derived from ancestors with a haploid chromosome number of six. *B. oleracea* ($n = 9$) has been examined to find out in how far expectation was fulfilled in this species. The material has proved suitable for the investigation of several problems relative to secondary pairing, viz. (1) its origin, persistence and disappearance; (2) its variation in different pollen mother-cells; (3) the relation between degrees of secondary pairing at metaphase I and metaphase II; and (4) the relation between the degree of secondary pairing in the two plates at metaphase II. Data upon all these problems would yield information about the mechanics of secondary pairing and the movements of the chromosomes relative to one another during meiosis.

I am very greatly indebted to Dr. K. Mather, of the Galton Laboratory, University College, London, for making the calculations and also for valuable advice concerning the statistical treatment.

Material and Methods

The material consists of several wild strains of *B. oleracea*, raised from seed collected at Kingsdown, Kent and Babbacombe, Devon, together with plants of Sutton's Drumhead Borecole. Root-tips were fixed in the modified Flemming previously used on similar material (Catcheside, 1934). Smears of pollen-mother-cells were also fixed in this modified Flemming and also in medium Flemming to which about 0.2% of maltose had been added. Root-tips were sectioned at about 16μ . All material was stained by the Newton iodine-gentian violet technique. All figures, except that of the somatic chromosomes (fig. 1), were drawn from smears of the Borecole, and are $\times 3,600$. No difference between the wild and the cultivated strains were noted; the numerical data were taken from three slides of Borecole.

Description

All the strains of *Brassica oleracea* examined have eighteen chromosomes in their somatic complements (fig. 1). At meiosis,

nine bivalents were regularly observed in all cells; no cases of multivalents or of univalents were seen.

At diakinesis, the nine bivalents are situated at the periphery of the nucleus such that the distances between neighbouring bivalents are approximately equal (fig. 2). The arrangement is such that

Figs. 1-18 *Brassica oleracea*. Fig. 1 Somatic metaphase plate. Fig. 2 Pollen mother-cell at diakinesis. Figs. 3-18 Pollen mother-cells at metaphase I, in polar view, showing types of secondary pairing. Fig. 3 No secondary pairing; figs. 4-6 one group of two bivalents; figs. 7-11 two groups of two bivalents each; figs. 12-15 three groups of two bivalents each; fig. 16 four groups of two bivalents each; fig. 17 one group of three bivalents; fig. 18 one group of three, and two groups of two, bivalents.

each bivalent has a number of neighbours and a complementary number of non-neighbours. The mean number of neighbours is about five. The nucleolus is also peripheral. The equidistant peripheral arrangement of the bivalents indicates that there is an approximately equal force of repulsion between all the bivalents. At late diakinesis, the repulsion appears to weaken. At any rate the distances between some bivalents diminishes and this continues during prometaphase until finally there are often one or more groups of secondarily paired bivalents. The general behaviour is consistent with the supposition that bivalents which are paired secondarily at the end of prometaphase have occupied neighbouring positions

at diakinesis. There does not seem to be any change in the relative spatial dispositions of the bivalents between diakinesis and prometaphase apart from the closer approximation, especially of some of them. It will be seen that this is borne out by the statistical data.

With the onset of full metaphase, the inter-bivalent repulsions increase so that the clustered group seen at prometaphase gradually gives place to a level plate in which bivalents, or groups of secondarily paired bivalents, are more spread out. A summary of the observed kinds of secondary pairing, recorded from three slides, is given in Table 1; drawings of the different kinds are given in

Table 1. Frequencies of secondary pairing types at metaphase in pollen mother-cells of *Brassica oleracea*

Type of metaphase plate	Numbers observed							
	Metaphase I				Metaphase II			
	Slide 1	Slide 2	Slide 3	Totals	Slide 1	Slide 2	Slide 3	Totals
9(1)	14	7	11	32	0	3	8	11
1(2)+7(1)	32	36	35	103	2	20	26	48
2(2)+5(1)	51	39	32	122	5	46	27	78
3(2)+3(1)	41	23	16	80	4	24	14	42
4(2)+1(1)	3	0	1	4	0	1	1	2
1(3)+6(1)	2	1	6	9	0	2	2	4
1(3)+1(2)+4(1)	6	7	6	19	1	11	3	15
1(3)+2(2)+2(1)	3	4	3	10	1	9	1	11
1(4)+5(1)	3	2	0	5	0	1	0	1
2(3)+3(1)	0	0	1	1	0	2	0	2
2(3)+1(2)+1(1)	0	0	0	0	0	1	0	1
Grand Totals				385				215

figs. 3–18. The observed kinds of secondary pairing at metaphase I fall into ten classes and at metaphase II into eleven classes. An inspection of the frequencies of the various classes suggests that the first four are normal and characteristic, and the remainder aberrant. The data agree well with the hypothesis (Catcheside, 1934) that the basic chromosome number in *Brassica* is six. The characteristic maximum shows three groups each of two bivalents, and three ungrouped bivalents. The characteristic classes of secondary pairing, those with none, one, two or three groups of two secondarily paired bivalents, show a unimodal variation, indicating that there is a definite law of chance governing the occurrence of secondary pairing. The aberrant classes (figs. 16–18) may be due to at least three causes: (1) inferior fixation leading to clumping and false secondary association; (2) mistaken observation; and (3) real structural complexity, especially reduplication, within the complement, in addition to the secondary polyploidy. As will be seen later, the homogeneity of the data shows that the first two causes cannot

play an important part. In conformity with this view, also, is the observation that occasionally bivalents of unlike size are secondarily paired (note figs. 4, 6, 10 and 17). This is confirmed by observations of similar cases in side view, when it is possible to see that there is no difference between the two bivalents, in regard to numbers of chiasmata or configuration, such as would lead to an apparent size difference when in polar view.

At metaphase I, in the absence of secondary pairing, the nine bivalents are usually grouped two central and seven peripheral (fig. 3). Less commonly, there are one central and eight peripheral bivalents. With a single secondary pair and therefore eight independent groups, two central bivalents are still the commonest arrangement. The two bivalents of the secondary pair may be central (fig. 4) or peripheral and in the latter case either radial (fig. 5) or tangential (fig. 6) with respect to the plate. With fewer independent groups than eight, and therefore more secondary pairs, there is more frequently a single group in the centre of the plate. This group is usually a single bivalent but may be a group of two, or of three, bivalents. The different groups in the metaphase plates when two or more secondarily paired groups are present, are arranged with respect to one another in all conceivable ways, presumably at random though this is hard to test in practice. More than sixty metaphase patterns, differing in numbers and relative arrangements of secondarily paired groups, have been observed in the present material. The observations, however, cannot be conveniently summarised, the grouping of many of them is highly subjective and no valuable analysis of the data as a whole presents itself. The classification has therefore been omitted.

No cases of multivalent primary association have been seen. This indicates that homology of the dissimilar, secondarily pairing, chromosomes must be intermittent and only over short distances at a time.

Primary association is usually by terminal, rarely by interstitial chiasmata. The chromosomes therefore disjoin synchronously at anaphase I and move to the poles in almost flat plates. With the commencement of anaphase I, repulsion between the secondarily paired bivalents sets in intensely so that by mid-anaphase I nearly all secondary pairing has disappeared. It is completely gone by telophase I. Polar views of anaphase I plates have about the same area as metaphase I plates (cf. figs. 19 and 29 with earlier figs.). The two anaphase I plates in a pollen mother-cell correspond in the arrangement of their chromosomes. There is no movement of the chromosomes relative to one another at anaphase

except for that producing uniform spacing. Chromosomes that were secondarily paired at metaphase retain adjacent positions during anaphase and pass into the telophase nucleus as neighbours.

Figs. 19-20 *Brassica oleracea* pollen mother-cells at anaphase I, in polar view, showing the loss of secondary pairing.

The telophase I nucleus is constructed from the flat anaphase plate and therefore it is at first nearly flat or, strictly, concavoconvex, the concavities of the two

telophase nuclei of one pollen mother-cell being apposed (fig. 21). The chromosomes are equidistant in one plane; as seen in polar view

Figs. 21-26 *Brassica oleracea* pollen mother-cells at interphase stages. Figs. 21 and 22 are early telophase I in side and polar views respectively showing the flat nucleus and uniform distribution of chromosomes. Figs. 23-25 are interphase nuclei changing to a spherical shape. Fig. 26 shows prometaphase II with some cases of secondary pairing.

(fig. 22), their arrangement is like that of a metaphase I plate that lacks secondary pairing. During interphase the nucleus expands axially, remaining the same width transversely. At the same time, the chromosomes spread around the periphery (figs. 23 and 24) to give a diakinesis-like arrangement. It is likely that there is some movement of the chromosomes relative to one another. But it is impossible to determine directly

Figs. 27-30 *Brassica oleracea* pollen mother-cells at metaphase II showing secondary pairing types.

Table 2. Correlation between secondary pairing in the two metaphase II plates in pollen mother-cells of *B. oleracea*

Type of Plate A	Type of Plate B							
	9(1)	1(2)+7(1)	2(2)+5(1)	3(2)+3(1)	4(2)+1(1)	1(3)+6(1)	1(3)+1(2)+4(1)	1(3)+2(2)+2(1)
9(1)	1	4	1			1		
1(2)+7(1)		6	11	1		1	3	
2(2)+5(1)			10	14			3	2
3(2)+3(1)				4	2		1	2
4(2)+1(1)								
1(3)+6(1)								
1(3)+1(2)+4(1)								3
1(3)+2(2)+2(1)								2
Variance	Degrees of freedom		Sum of Squares		Mean Square		Log. Mean Square	
Between pollen mother-cells	71		345.3264		4.9905		1.60756	
Within pollen mother-cells	72		148.5000		2.0625		0.72393	
	sum 143		sum 493.8264				Diff. 0.88363	

how much of such movement occurs. A correlation in the degree of secondary pairing shown by the two metaphase II plates could be taken to indicate a minimum of movement.

The split in the chromosomes becomes obvious towards the end of interphase (fig. 25), the nuclear membrane disappears and pro-metaphase II follows (fig. 26). Again the chromosomes approach one another and some become secondarily paired. Metaphase II plates (figs. 27-30) show various degrees of secondary pairing, covering the same range of types as at metaphase I, and in similar proportions. The frequencies of the different types in the three slides are recorded in Table 1. In the case of 72 pollen mother-cells, both metaphase II plates were observed in polar view. These are specially recorded in Table 2 to determine whether there is any correlation between them.

Statistical Analysis

Tests of Homogeneity

For this purpose, the data of Table 1 have been simplified in Table 3 by combining all the allegedly aberrant classes of secondary pairing, namely classes 5 to 11 of Table 1. The simplified table may then be treated as a contingency table and the contributions to χ^2 for the different degrees of freedom obtained.

Table 3. Test of homogeneity of metaphase I and II observations

Class		Metaphase I				Metaphase II				Total MI and MII
		Slide 1	Slide 2	Slide 3	Total	Slide 1	Slide 2	Slide 3	Total	
9(1)	obs. calc.	14 12.8831	7 9.8909	11 9.2260	32 27.5917	0 0.6651	3 6.1395	8 4.1953	11 15.4083	43
1(2)+ 7(1)	obs. calc.	32 41.4675	36 31.8364	35 29.6961	103 96.8917	2 2.9023	20 26.7907	26 18.3070	48 54.1083	151
2(2)+ 5(1)	obs. calc.	51 49.1169	39 37.7091	32 35.1740	122 128.3333	5 4.7163	46 43.5349	27 29.7488	78 71.6667	200
3(2)+ 3(1)	obs. calc.	41 32.2078	23 24.7273	16 23.0649	80 78.2833	4 2.5395	24 23.4419	14 16.0186	42 43.7167	122
Aber- rant	obs. calc.	17 19.3247	14 14.8364	17 13.8390	48 53.9000	2 2.1767	27 20.0930	7 13.7302	36 30.1000	84
Totals		155	119	111	385	13	120	82	215	600
			χ^2		Degrees of Freedom		Conclusion			
Within metaphase I			9.0364		8		No heterogeneity Some heterogeneity (P between 0.02 and 0.05)			
Within metaphase II			18.1619		8					
Between M I and M II			5.8197		4		No heterogeneity			

The calculated values of m , the expected value in a perfect sample, for columns 3-5 in Table 3, viz. metaphase I frequencies for each slide, were obtained by dividing the observed marginal totals for metaphase I in column 6 between the three slides in the proportions of the total metaphase I observations for each slide, viz. the total figures at the feet of columns 3-5. The calculated values in columns 7-9 were obtained in a like manner from the totals in column 10 and the totals at the feet of columns 7-9. The calculated values in columns 6 and 10 were obtained by dividing the totals in column 11 in the proportions of the number of observations made from all three slides at each division. The contribution to χ^2 were then calculated with a minimum of labour, using the formula $\chi^2 = S \left(\frac{a^2}{m} \right) - n$; this is a transposition of the usual formula $\chi^2 = S \left(\frac{a-m^2}{m} \right)$. As shown at the foot of Table 3, there is a slight indication of heterogeneity in the metaphase II observations. The contribution of the aberrant classes to the metaphase II χ^2 is 6.8315 for 2 degrees of freedom, and lies between 0.02 and 0.05; the remaining classes therefore contribute 11.3304 for 6 degrees of freedom and P for them lies between 0.05 and 0.10, we may therefore conclude that the data in Table 3 are homogeneous and that any slight tendency to heterogeneity may be traced to the aberrant classes, a group to which some doubt already attaches for quite other reasons.

We may also make an analysis of χ^2 in the same way for the normal pairing classes alone. Table 4 shows the values of this statistic for the sources of variation important for our purpose.

Table 4. Test of homogeneity for the normal classes of secondary pairing alone.
(Cf. Table 3)

Interaction (Source of variation)	χ^2	Degrees of freedom	P
Divisions (I and II) and Secondary Pairing Types	3.7795	3	0.30-0.40
Slides (1, 2 and 3) and Types	16.1349	6	0.01-0.02 significant
Divisions, Slides and Types	3.8834	6	0.50-0.70
Totals	23.7978	15	

There is a significant difference between the slides which may be traced to the following possible causes: (1) differences in fixation leading to slight clumping in some cases; (2) differences in subjective counting of doubtful cases at different times. The latter possibility may be examined as the material on each slide was counted

over a period of several days. A test of the interaction between secondary pairing type and day of scoring failed to disclose any heterogeneity traceable to this cause.

Test of Metaphase II Correlation

We may also determine whether the degree of the secondary pairing in the two plates (cf. Table 2) at metaphase II tends to be similar. This is best done by analysing the variance into two parts between and within pollen mother-cells. This is done at the foot of Table 2. There is no question that the difference between the two mean squares is significant. The value of z is 0.44182 which is well outside the 1% probability given in Fisher's (1928) Table IV. Hence there is less variation within than between the pollen mother-cells and therefore a positive correlation between the kinds of secondary pairing in the two metaphase II plates of a pollen mother-cell. This means that the chromosomes at late interphase are not arranged at random but are influenced in their arrangement by that of the previous metaphase I. Therefore there is little movement of the chromosomes relative to one another at interphase. Such movement that does occur is probably all due to the change from the flat plate of telophase to the hollow sphere of interphase. There is little doubt that interphase, like the resting stage of mitosis, is in truth a resting stage mechanically.

Likelihood of Secondary Pairing

It has already been suggested that secondary pairing is dependent upon the bivalents lying or failing to lie in positions where they may pair at prometaphase. A parameter, the mean chance of two bivalents pairing, may be calculated from the observed frequencies in column 11 of Table 3, neglecting the aberrant classes. The aberrant classes could be included, but the meaning of the parameter estimated would be less definite and more open to doubt than that estimated in the present way. It is assumed: (1) that three pairs of bivalents are each capable of pairing secondarily; (2) that each pair has an equal chance, q , of pairing secondarily and a chance, p , of failing so to pair, such that $p + q = 1$. The frequencies of metaphase plates with 0, 1, 2 and 3 groups of secondarily paired bivalents would be given by the expansion of $(p + q)^3$, namely $p^3 + 3p^2q + 3pq^2 + q^3$. The best values of p and q may be estimated from the data by the method of maximum likelihood (cf. Mather 1936). Putting $q = 1 - p$, the logarithm (L) of the likelihood expression is

$$L = 43 \log p^3 + 151 \log 3p^2(1-p) + 200 \log 3p(1-p)^2 + 122 \log (1-p)^3.$$

This expression must be maximised; this condition will be satisfied by differentiating with respect to p and equating the differential to zero. The resulting equation

$$\frac{dL}{dp} = \frac{129}{p} + \frac{151(2-3p)}{p(1-p)} + \frac{200(1-3p)}{p(1-p)} - \frac{366}{1-p} = 0$$

may then be solved for p by successive approximation and linear interpolation (cf. Mather, 1936, p. 406). The standard error of the estimate is directly obtainable from the approximations. The values calculated are $p = 0.4077 \pm 0.0129$ and $q = 0.5923 \pm 0.0129$; the value of q is the chance that any particular secondary pair will occur.

The degree to which the total observed frequencies of secondary pairing follow a binomial distribution is estimated in Table 5, using the above values of p and q in calculating the expected numbers in each class. The value of χ^2 is 5.9640 for 2 degrees of freedom,

Table 5. Test of fit of normal secondary pairing frequencies to a binomial distribution

Type of metaphase plate	Observed numbers	Expected numbers	$\frac{a^2}{m}$
9(1)	43	34.9683	52.8765
1(2)+7(1)	151	152.4037	149.6092
2(2)+5(1)	200	221.4089	180.6612
3(2)+3(1)	122	107.2196	138.8179
Totals	516	516.0005	521.9648

$$\chi^2 = S \left(\frac{a^2}{m} \right) - n = 521.9648 - 516 = 5.9648.$$

Degrees of freedom = 2; χ^2 at 5% point = 5.991; difference barely significant.

namely four degrees of freedom less one for the total number of observations and one for the estimated parameter (p or q). The value of χ^2 at the 5% point for 2 degrees of freedom is 5.991, so the difference is barely significant. The distribution of the differences is peculiar, inasmuch as the lowest and highest values of secondary pairing are too frequent relative to the intermediate values. The mode is not displaced towards a high or lower frequency as might be expected. Errors may have crept into the observed frequencies from various sources: (1) A not inconsiderable class of aberrant metaphases have been omitted and these necessarily consist mainly of those with an intermediate degree of secondary pairing; further the omission of these aberrant classes has probably lowered the estimated value of q . (2) There is a tendency for adjacent metaphase plates to be correlated at metaphase II; presumably a similar effect holds at metaphase I. It would contribute to distortions of the normal distribution, particularly in small samples.

Discussion

On the whole the data indicate that the degree of secondary pairing closely follows a normal binomial distribution. This can be related to a simple mechanical basis.

At diakinesis, bivalents are arranged at random around the periphery of the nucleus. Each one is surrounded by a number of neighbours, the balance of the bivalents being non-neighbours. In the present instance, each bivalent is surrounded by about five other neighbour bivalents, there being three non-neighbour bivalents. Hence each bivalent has a chance, five-eighths (0.625), of lying adjacent to a similar bivalent inside the diakinesis nucleus, there being two of each kind, and therefore in a position to pair secondarily at prometaphase. This value accords remarkably well with the estimated value of q (0.5923 ± 0.0129) with which it corresponds. We may conclude that secondary pairing is solely dependent upon the diakinesis positions of bivalents relative to one another and therefore that all bivalents which lie adjacent at diakinesis and which are capable of secondary pairing are so paired at metaphase I. Similar considerations would seem to apply to interphase and metaphase II and to prophase and metaphase of mitosis.

The hypothesis is capable of test in the following manner, bearing in mind our two untested assumptions (1) that all chromosomes or bivalents repel one another equally at the end of prophase and (2) that the power of attraction between similar chromosomes (or bivalents) is equal for the whole complement. For, in certain cases, e.g. for 4, 6, 8, 12 etc. bodies (chromosomes or bivalents) it is easy to deduce theoretical late prophase (or diakinesis) arrangements of the bodies from geometrical analogies. The numbers of neighbour and non-neighbour bodies per body for several cases, are shown in Table 6, together with the values of q , the chance of two particular

Table 6. Theoretical chance of secondary pairing between two bodies in nuclei with a specified number of bodies

Number of bodies	Number of neighbours per body	Number of non-neighbours per body	Value of q , i.e. chance of two similar bodies lying adjacent
4	3	0	1.0
6	4	1	0.84
8	3 close, 3 distant	1	0.857 (maximum)
12	5	6	0.455

(i.e. similar) bodies lying adjacent. These values could be tested on suitable material.

In the absence of movement of chromosomes relative to one another during the resting stage, the late prophase pattern would

be that adopted at the previous telophase. This is important in *Drosophila*, since Kaufmann (1934) has shown that the chromosomes are somatically paired during prophase. Here the telophase pattern would be the determiner of somatic pairing.

Secondary pairing, and somatic pairing, is subject to limitation by several conditions the actions of which are by no means understood though capable of more exact study. (1) Large size of the chromosomes appears to inhibit it, since it is seen only in organisms with small chromosomes. (2) Numerical differences cause changes in frequency, high numbers of chromosomes reducing the chance of association between similar chromosomes. It would be instructive to compare somatic pairing in diploid and triploid *Drosophila*. (3) Structural dissimilarity, presumably, will weaken the attraction and may finally reach a point at which the attractive force is inadequate. Compare, for instance, the low frequency of secondary pairing in cultivated pears with its high frequency in cultivated apples (Moffett, 1934). (4) An exceptionally high force of repulsion at the centromere might inhibit pairing. (5) Inhibition of secondary pairing may be genetically controlled; no clear cases of such racial differences are yet known. In this connection, it should be possible to determine the relation between primary and secondary pairing by comparison of asynaptic with normal types.

Summary

1. Observations upon secondary pairing of bivalents at meiosis in *Brassica oleracea* ($n = 9$) suggest that this species is a secondary polyploid with a basic chromosome number of six. Three of the six basic chromosomes are present in duplicate, the other three but once each. Structural changes, inducing aberrant kinds of secondary pairing, are superimposed.

2. The course of meiosis subsequent to diakinesis is described and it is shown that there is no direct continuity between the secondary pairing present at metaphase I and metaphase II respectively, though the former tends to determine the latter.

3. The relative frequencies of different types of secondary pairing at metaphase I and II are similar. Statistical tests indicate that any heterogeneity inherent in the data lies between the different preparations examined; probably fixation can cause minor changes, especially emphasis of weak secondary pairing.

4. There is a significant correlation in type of secondary pairing between the two metaphase II plates of a pollen mother-cell. Probably no movement of the chromosomes relative to one another

occurs during interphase, except during the period when the spherical distribution of the chromosomes is being established.

5. In the normal classes of secondary pairing two similar bivalents have a mean chance, 0.5923 ± 0.0129 , of pairing secondarily. This is close in value to the chance, about 0.625, of two particular bivalents lying adjacent at diakinesis. The normal classes fit a binomial distribution fairly well.

6. We may conclude that two similar bivalents will be paired secondarily at metaphase I if they occupy adjacent positions at diakinesis. Similar data apply to interkinesis and metaphase II and to prophase and metaphase of mitosis. The mechanism, however, will always be subject to the limitations imposed by mechanical and genetical inhibitors.

Bibliography

- Alam, Zafar, 1936. Cytological studies of some Indian oleiferous Cruciferae. *Ann. Bot.* 50: 85-102.
- Catcheside, D. G., 1934. The chromosomal relationships in the swede and turnip groups of *Brassica*. *Ann. Bot.* 48: 601-633.
- Fischer, R. A., 1928. *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh.
- Kaufmann, B. P., 1934. Somatic mitosis of *Drosophila melanogaster*. *J. Morph.* 56: 125-155.
- Mather, K., 1935. The combination of data. *Ann. Eugenics* 6: 399-410.
- Moffett, A. A., 1934. Cytological studies in cultivated pears. *Genetica* 15: 511-518.
-