On the Nature of Chromosome Association in *N. tabacum* Haploids

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The meiotic behavior of chromosomes in haploids is of great theoretical interest because of its bearing on problems of synapsis and chromosome homology. Of the numerous accounts of meiotic behavior in haploids to be found in the literature perhaps the most thoroughgoing is that of Catcheside (1932) which deals with a haploid *Oenothera*. While the seven chromosomes usually fail to pair at diakinesis and metaphase occasional multivalent associations of definite types were discovered, mostly rod bivalents and Y or V shaped trivalents involving terminal, subterminal and interstitial chiasmata. These associations may logically be considered good evidence for the existence of short homologous segments in different chromosomes of the set under the following assumptions:

(1) That any pairing between chromosomes depends upon individual attraction of like particles or chromosomes and conversely unlike particles do not pair. Allelomorphic genes being unlike are assumed not to pair, but to be brought together mechanically owing to the homologies of the neighboring genes.

(2) That pairing occurs at zygotene between any homologous segments of the chromosomes no matter how short they may be and

(3) That chiasmata terminalize only to the distal end of homologous segments; i.e., when chromosomes become different in their homologies over a considerable distance a chiasma terminalizing in the homologous region will be prevented from moving beyond the end of the segment.

Though *a priori* the above assumptions may seem very reasonable, they should nevertheless be subjected to the test of direct observation if possible. The only data so far presented which bear on this question are those of McClintock (1932) on the pachytene behavior of trisomic and B-type chromosomes in *Zea mays*. In this material there is clearly occasional non-homologous association of chromosomes at pachytene,

the pairing being apparently just as intimate as that between homologous parts. Although apparently only rarely leading to the production of chiasmata, these observations do definitely establish the fact that pairing of chromosomes is not necessarily always due to individual attraction of like particles or chromomeres. The following studies of pachytene and metaphase behavior in three haploids of *N. tabacum* are of interest as regards the question of the validity of interpreting the occasional associations seen in haploids and many hybrids as evidence of duplication of segments.

The Coral *N. tabacum* Haploid

The origin of coral *tabacum* and its dependence upon genetic modification of the F chromosome has been described by Clausen (1931). More recent cytological observations on microsporocytes have disclosed the fact that coral exhibits quadrivalent associations ($22_{II} + 1_{IV}$) in about fifty per cent of the cells. These and certain other studies have led to the conclusion that coral is a translocation product involving replacement of a portion, or more probably practically the entire arm of the F chromosome, with a segment of approximately equal size from some as yet unidentified chromosome. In terms of this conception normal *tabacum* may be represented by the general formula $(22 + aFb + cXd)_{II}$, and coral by $(22 + aFc + cXd)_{II}$, (a, b and c, d) representing the arms or parts of the arms of the F and the unidentified X chromosomes. Coral is then deficient for part of the b arm and duplicate for part of the c arm.

In a population obtained by crossing coral ♀ × *N. tomentosiformis* ♂ a haploid plant was found which was coral in flower color and also resembled the coral type in leaf and growth habit. The normal *N. tabacum*, although theoretically amphidiploid in origin, regularly forms 24_{II} at I-M in the diploid and the haploid has hitherto been considered to exhibit only univalents in the meiotic divisions. Accordingly as the diploid coral type showed quadrivalents at I-M, the haploid coral was expected to show a large number of cells with one bivalent. It was also hoped that pachytene studies would reveal the extent of the translocated duplicate segment. Instead an unexpectedly large number of bivalents was found in many cells. The I-M observations are summarized in Table 1. The slides were made according to the usual aceto-carmine smear technique using unfixed material.

The associations on the spindle were usually bivalents with a single chiasma though occasional trivalent configurations were observed. Only those cells were recorded as exhibiting associations where the connections between the chromosomes were definitely chromatin in

nature. The number of recorded bivalents therefore is probably lower than the actual number for many associations with only threadlike connections were omitted because of reasons to be presented later.

Figs. 1-6. Diakinesis and metaphase associations in *N. tabacum* haploids. **Fig. 1.** Diakinesis of the coral haploid showing four fragments. Note the long fibres extending from each chromosome often connecting one with another. **Fig. 2.** I-M of coral haploid showing $3\text{II}+18\text{I}+1$ fragment. The bivalents have only one chiasma. **Fig. 3.** I-M of the normal haploid showing 24 univalents. Note the "off spindle associations" connecting many of the chromosomes. **Fig. 4.** Bivalents found in the normal haploid. *a* and *b* are held together by terminal chiasma; *c-f* by subterminal and interstitial chiasmata. In the coral haploid most of the bivalents are comparable to those of *c-f*. **Fig. 5.** Various fragments found in the normal haploid. **Fig. 6.** Polar view of a coral haploid cell at I-M showing maximum association of $6\text{II}+12\text{I}$.

The types of bivalents found in these cells are shown figures 2, 6, 7 and 8. Figure 6 is a polar view of one of the few extreme instances of maximum association. In this cell the bivalents appeared comparable to those found in the normal diploid. No attempt was made to determined the exact nature of the chiasmata connecting the univalents; i.e. how many were terminal, subterminal, or interstitial. However, many were plainly terminal and there seemed all gradations

Figs. 7 and 8. Side views of I-M in *N. tabacum* coral haploid showing varying number of bivalents. Note the three fragments in figure 8.

Table 1. I-M cells of the coral haploid showing distribution and number of associations on the spindle.

Number of bivalent associations	0	1	2	3	4	5	Total
Slide 1	33	41	46	11	5	1	137
„ 2	4	11	10	2	0	0	27
„ 3	2	23	7	3	0	0	35
„ 4	3	11	10	7	2	0	33
„ 5	12	8	8	4	1	2	35
Total	54	94	81	27	8	3	267

The average number of bivalents per cell is 1.44.

between these and what Catcheside, Darlington and others usually consider interstitial chiasmata.

Another unexpected and therefore very interesting feature was the presence of an unusually large number of fragments in these cells. Thus of the 267 cells recorded in Table 1, 78 had one fragment, 35 had two fragments, 8 had three fragments and one had four fragments. The significance of these fragments will be discussed later after a description of pachytene association has been given.

The Normal *N. tabacum* Haploid

The puzzling series of phenomena above described necessitated a reexamination of the normal haploid. Fortunately this was immediately possible because of the chance appearance of what seemed morphologically a haploid in the progeny of a monosomic type crossed with *N. tabacum*. Cytologic study showed it to have 24 univalents at I-M. Though usually only univalents were found occasional cells showed bivalent associations, as shown by the I-M observations recorded in Table 2. In Table 3 similar data is given for another normal haploid found in the progeny of a monosomic type crossed to *N. tomentosa*. This culture was growing in the field during the summer of 1933 under obviously different environmental conditions from those obtaining in the greenhouse during the winter and early spring when the observations on the other haploids were made.

The bivalent associations observed in both normal haploids were similar to those found in the coral haploid except that many were of the type described as terminal chiasmata; i.e., were univalents connected only by very thin strands of chromatin as shown in figure 4*a* and *b*. The number of bivalent associations having what are usually called subterminal and interstitial chiasmata (Fig. 4*c-f*) was relatively much less frequent. The average number of associations per cell was .43 in the winter grown and .17 in the summer grown haploid. Evidently we must recognize some variability in the amount of pairing due presumably to environmental differences for the two haploids are of identical genetic constitution. The maximum amount of association found is, however, distinctly less than that found in the coral haploid, namely, 1.43. It is of course impossible to determine exactly how much of this difference is due to the presence of the duplicate coral segment. Certainly the greater amount of association observed cannot all be due to chiasma formation in this segment for the number of cells without association recorded in Table 1 indicates that in at least 20% of the cases the coral segments fail to form effective chiasmata. Actually the coral segments must form bivalents in even less than 80% of the cases for

Table 2. I-M cells of a normal haploid grown in the green-house in the spring of 1933 showing the distribution and number of associations on the spindle.

Number of associations	0	1	2	3	4	5	Total
Slide 1	51	22	11	4	0	0	88
„ 2	160	43	16	3	0	0	222
„ 3	54	18	4	1	0	0	77
Total	265	83	31	8	0	0	387

The average number of bivalents is .43

Cells with one fragment—25

Cells with two fragments—3

Cells with three fragments—1

Table 3. I-M cells of a normal haploid field grown in the summer of 1933 showing distribution and number of associations on the spindle.

Number of associations	0	1	2	3	4	5	Total
Slide 1	394	57	14	2	0	0	467
„ 2	453	56	17	2	0	0	528
Total	847	113	31	4	0	0	1095

The average number of bivalents per cell is .17

Cells with one fragment—41.

many cells exhibit bivalents only because of association of other chromosomes. Hence it would seem that theoretically one would expect the coral haploid to show an average association considerably less than 1.23 bivalents per cell (.43+.80) instead of the observed 1.44. The further consideration of the appearance of the bivalents and the distribution of associations adds to the impression that some other factor must be operating to increase association.

Besides the above bivalents on the spindle many cells had univalents which were connected to one another by one or two very fine threads. These associations seemed to be located at random in the cell and often most of the chromosomes seemed so connected. In fact the various slides differed markedly as respects these off-spindle associations, some showing very few such cells, each with only a few

univalents connected, and others having practically every cell with many of the chromosomes connected to one another. Often as many as five chromosomes were connected in tandem as shown in figure 3. Obviously such a series of connections cannot indicate five terminalized chiasmata and furthermore as the various slides differed so markedly in this respect it was considered that these connections had no relation to pairing and chiasma formation. A suggestion as to their origin will be given later.

A Comparative Study of the Pachytene Stage in the Normal and Coral Haploids

In view of the difference in behavior of the normal and coral haploids at I-M it was of interest to study the pairing relations at pachytene in order to see if the bivalents observed at metaphase were the result of pairing of long homologous segments duplicated in the two sets of chromosomes.

For pachytene studies a modification of McClintock's technique (1929) was used. The anthers were first fixed in 3 parts absolute alcohol and 1 part acetic acid. A part of one anther was then placed on a slide with a drop of aceto-carmin and the pollen mother cell tissue separated from the surrounding tissue by fine needles. A cover slip was then placed on the slide and a gentle pressure exerted while observing the cells under low power. In this way the cell walls could be broken and the protoplast floated free, thus allowing the chromatin threads to be flattened out and more clearly observed as to chromomere structure.

In Table 4 the number of associations at pachytene is given. The cells were carefully studied with special regard to the number and length of paired threads as well as to their homology as revealed by the degree of similarity in chromomeres of parts associated. Care was taken to get as nearly comparable stages in pachytene for both haploids as possible.

Table 4. Number of paired threads at pachytene stage in the cells of the coral and normal haploid.

Plant type	Number of cells with indicated number of paired threads									Total cases
	0	1	2	3	4	5	6	7	many	
Coral haploid	3	17	14	11	8	2	2	2	4	63
Normal haploid	83	22	8	3	0	0	0	0	0	116

Perhaps the most striking feature of this table is the far greater amount of association exhibited by the coral haploid. The difference actually is even more striking than that disclosed by the table, for in the normal haploid the lengths of the paired segments generally were much less than that of those found in the coral haploid. Although many slides were examined at all stages of pachytene even those where most of the cells had already passed into early diplotene failed to show cells with the amount of pairing regularly found in the coral haploid. Hence it is certain that the above differences cannot be merely due to a difference in the stage of meiosis.

Even more interesting was the difference in type of pairing. In the coral haploid many cells showed a long paired thread which seemed homologous throughout most of its length (figure 9) What is believed to be the spindle fibre region is shown at the arrow. It should be noted that to the right of the region the chromomeres seem homologously paired, while to the left they are not matched. It seems possible that this segment is the translocated duplicate coral segment, the break having occurred near the spindle fibre region. Besides this long paired segment which was present in many of the cells there were often other long regions paired non-homologously in part at least. Figure 10 shows one of these; the chromomeres in part at least are not matched. Figure 14 is a

Figs. 9-15. Pachytene associations in *N. tabacum* haploids. Fig. 9. Pachytene of the coral haploid showing a long segment paired homologously on the right side of the spindle fibre region (indicated by arrow) and apparently non-homologously on the left side. This is possibly the duplicated coral segment. Fig. 10. Typical pachytene association in the coral haploid. Many chromomeres are not opposed at all by partners. Fig. 11. Typical pachytene association in the normal haploid. Only short portions are usually paired. Fig. 12. A configuration simulating the tripachytene association of triploids. From the coral haploid. Fig. 13. Ring configuration found in the coral haploid. Figs. 14 and 15. Foldbacks often found in the coral haploid. Note that in figures 6 the chromosomes are not morphologically matched through intimately paired.

chromosome folded back on itself in which the chromomeres could be very clearly seen. Although closely associated there is nevertheless not a single chromomere with a morphologically similar mate. In fact in some parts the chromomeres are not opposed at all. Figure 15 is another more complex fold back in which one end is paired with a part of another chromosome. Here also the chromomeres near the folded end at least are not morphologically matched. In figure 12 a very unusual association of three strands is shown, with the conjugation apparently as intimate as that exhibited by the tripachytene associations so often found in the triploid *N. tabacum* (Olmo, 1934). Finally in figure 13 a ring configuration is shown, which is doubtless the result of a univalent's folding completely back on itself with the ends pairing together in the middle of the ring. Several of these ring structures were found. Occasionally also T-shaped configurations involving three different chromosomes were observed. In these configurations two strands intimately paired for a portion of their length separate and continue on in association with a third strand.

In the normal haploid the long homologously paired threads so often seen in the coral were not found. In fact very few threads were ever associated for any considerable length and in this respect the pachytene picture differed strikingly from that found in the coral haploid. Fold backs were occasionally found and also many configurations such as shown in figure 11. The pairing seemed mostly non-homologous as judged by the morphologic study of the chromomeres.

Discussion

Perhaps the most unexpected feature of this study is the non-homologous association of threads at pachytene, and especially the far greater extent to which it occurs in the coral haploid.

According to Catcheside, Darlington and others, as noted above occasional associations are to be referred to chiasma formation between homologously paired segments. Now as *N. tabacum* is theoretically an amphidiploid species it might readily be suspected that many of the chromosomes in the two sets contain duplicate segments with similar chromomeres and gene arrangement. Hence, some bivalents would be expected in the haploid and examination of the pachytene stage should reveal the existence of these duplicate segments. As has been described, however, the normal haploid showed very little homologous association at pachytene, most of the paired segments being short and plainly non-homologous as judged by their chromomeres. Furthermore, the coral haploid not only showed another long homologously paired thread which possibly represents the duplicated segment assumed

to be present in it but also many other long sections which were in part at least non-homologously paired as well as fold backs and terminally paired parts; all in far greater frequency than in the normal haploid.

It may perhaps be argued that many of these associations are due to the individual attraction of like particles duplicated in the chromosomes involved and that the other chromomeres are merely brought close together owing to the homologies of the neighboring genes as postulated by Catcheside (loc cit). In many cases this cannot be denied as the chromomeres in the paired regions were usually not sufficiently clear to decide as to their homology. However, in the case of the fold backs shown in figures 14 and 15 such was definitely not the case for as may be seen there were no morphologically similar chromomeres paired. Furthermore, McClintock (1932) working with much more favorable material has shown that a given chromosome in *Zea mays* may fold back on itself in different places.

It is difficult to imagine a system of duplicate segments which would allow such a complex type of homologous pairing. Rather it would appear that we must consider the homologous attraction of chromomeres as being in the nature of a regulatory mechanism in some way causing an orderly alignment of the threads when the cell as a whole is timed for synapsis. When true homologues are not present this tendency for two by two association of the chromatin threads expresses itself in part by non-homologous pairing. The evidence of Olmo (1934) in his study of triploid *N. tabacum* indicates that the ends of the chromosomes, pycnotic bodies such as large chromomeres or knobs, and spindle fibre regions serve as primary sources of attraction, the chromosomes being often associated at these points first and pairing then continuing along the chromosome from these points.

In the haploid then we occasionally get these pycnotic bodies, end bodies, and spindle fibre regions attracting each other non-homologously, and a certain amount of pairing results even though the chromomeres are non-homologous.

The greater frequency of pairing at pachytene observed in the coral haploid with the resultant possibly greater number of bivalents at I-M may then be due to a lengthening in the time factor allowing a greater amount of association to be formed at pachytene before the onset of diplotene stage. At least it cannot be said that the larger of associations is due to a greater number of duplicate segments other than the coral segment for the two strains originally came from the same pure line of *N. tabacum* var *purpurea*.

The fragments found in the haploids may be explained by assuming crossing over in the non-homologously paired segments and foldbacks. McClintock (1933) has evidence in *Zea mays* which suggests

that translocations occasionally occur in regions which are non-homologously associated. Thus association of the three chromosomes in trisomic plants frequently results in (1) a bivalent plus a univalent foldback or (2) a T-configuration. Since several cases of ring-shaped chromosomes and deleted rod-shaped chromosomes have arisen in the progeny of these plants it is suspected that they occurred as the result of translocations in a foldback univalent. Similarly in the *tabacum* haploids translocations (which are probably due to rare crossing over between non-homologous parts of chromosomes) in the foldbacks may occasionally give ring fragments. In the non-homologous paired segments such crossing over may also give fragments without spindle fibre regions and long chromosomes with two spindle fibre regions. The greater frequency of the fragments in the coral haploid is then due to the greater amount of non-homologous association found, especially foldbacks.

In view of the observed non-homologous pairing at pachytene we must interpret the bivalents found at I-M as the result of these pachytene associations continued on into the metaphase stage. It is of course understood that this conception is contrary to that usually held, namely that chromosomes are held together by chiasmata formed only between homologously paired parts and that they terminalize only until the distal end of the homologous segment is reached (Catcheside loc cit). However, as many of the observed bivalents have what are usually considered terminalized chiasmata it would appear that chiasma can either be formed in and terminalize through non-homologously associated segments or else some other mechanism is operating to continue the partial association of the chromosomes at pachytene on into the metaphase in the form usually considered as indicative of either interstitial or terminal chiasmata.

It would then seem probable that many of the diakinesis and I-M associations reported in haploids and hybrids such as those reported by Emerson (1928) and Catcheside (1932) in *Oenothera* actually do not indicate duplicate segments at all but are the result of non-homologous pachytene association similar to that found in the *N. tabacum* haploids. The seven chromosomes of *Oenothera* are rather similar in size at diakinesis and metaphase as indicated by the figures of Catcheside (loc cit) and it may well be that the various terminally associated bivalents observed are not actually the same ones each time. In fact even the critical figures of the unequal bivalent may merely be the result of any two chromosomes pairing at random in such a way that the end region of one is non-homologously paired with an interstitial region of the other. Crossing over would then give the same figures as those observed.

There is also the possibility that many so called bivalents seen in haploids and hybrids are actually nothing more than the "off spindle associations" so commonly found in some slides of the *N. tabacum* haploid. As before stated these can hardly be of any significance as regards previous association or indicative of homology. They are rather to be explained as fixation products; i.e., possibly strands of the enveloping chromatin matrix which at times are fixed in such a way as to be visible. Thus the diakinesis stage (figure 1) indicates that rather long fibres extend from practically every chromosome often connecting various univalents. It is believed that the off spindle associations so frequently observed in some slides are merely these strands continued on into the metaphase.

The position is not here taken that all the associations at I-M reported by various authors as occurring in haploids and species with a low frequency of variable pairing are the result of random non-homologous pachytene synapsis. Undoubtedly in some cases homology is a very important factor in their formation, as for example in the case of the *N. tomentosa-glutinosa* hybrid studied by Elvers (1934). In this case many of the pachytene associations were clearly homologous in nature. Accordingly, although the number of chromosomes is the same as in the haploid *N. tabacum* (24), we get a much higher average number of bivalents per cell at I-m. More critical comparative pachytene studies are needed before we can differentiate between those associations due to homology and those due to merely random non-homologous pairing. The differential behavior of the coral and normal *N. tabacum* haploids further indicates that a given haploid set of chromosomes may under certain physiologic conditions show a greater amount of non-homologous association. These varying physiologic conditions may be the result of either environmental or genetic differences or both. It would appear that any inference as to duplicate segments or homology which depends on observations as diakinesis or metaphase is very questionable when dealing with haploids or hybrids exhibiting occasional associations.

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Summary

1. Evidence is presented which indicates that a haploid of coral *N. tabacum* shows a higher degree of association at pachytene and I-M

than is found in normal haploids.

2. At the pachytene stage these associations are mostly non-homologous in nature except for the duplicate coral segment. T-shaped configurations and foldbacks are also found especially in the coral haploid and the associated sections are much longer than in the normal haploid.

3. As a result of these pachytene associations a variable number of rod-bivalents are found at I-M, the number being higher in the coral haploid. These bivalents have only a single chiasma which may be either terminal, subterminal or interstitial.

4. Fragments are found in both haploids but their frequency is much higher in the coral type, presumably because of the greater amount of non-homologous association. These fragments indicate crossing over has occurred in the non-homologously paired regions or in the foldbacks.

5. It is concluded that observation of bivalents at diakinesis and I-M in haploids and hybrids with variable pairing is not of itself proof of duplicate segments.

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