TELOMERE ATTACHMENT OF CHROMOSOMES. SOME GENETICAL AND CYTOLOGICAL CONSEQUENCES

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ATTACHMENT of chromosomes to the nuclear membrane has been suggested from cytological preparations on a number of occasions, e.g. by JANSSENS (1924) and DARLINGTON (1958), while UPCOTT (1939) has commented on the tendency for chromosome ends in Tulipa to be located near to the outside of the nucleus. In the present paper the possible consequences of a particular type of chromosomal attachment are considered. It is envisaged that homologous telomeres are attached together or in close proximity on the nuclear membrane. Of particular interest will be the manner in which such attachment may be expected to affect chromosome pairing in meiosis. No assumption is made about the relative position of the two telomeres of a chromosome, or of telomeres of different chromosomes, although evidence from "bouquet chromosomes" of some insect species (see WHITE 1954) indicates that in some species all ends may be in close proximity. It will be shown that evidence from a number of sources, particularly genetical and cytological evidence from tetraploids, is in agreement with predictions based on such attachment. Attention will mainly be directed to plant species, since as will be discussed later it seems possible that the attachment in animal species is considerably more complex.

Initiation of Chromosome Pairing

Until recently the only models of chromosome pairing discussed in any detail have been those where pairing is initiated following meeting of homologous chromosome regions either through long-range attraction or chance meeting. Failure to clarify the nature of such postulated long-range forces has reduced the value of the first hypothesis. The second hypothesis seems a little unlikely in view of the regularity with which pairing must be initiated in meiosis, even though it has been pointed out that there is comparatively little chromosome movement in the resting cell preceding the first division of meiosis (RHOADES 1961), and that the regularity of the chromosome configuration following the final premeiotic mitosis may tend to be preserved.

It is seen that the attachment of telomeres furnishes a ready basis for the initiation of pairing. Homologous distal regions of a chromosome would be brought in close proximity, especially when contraction occurred, and pairing could be initiated in these regions. This is consistent with the well documented observations of SCHRADER (1941) in the earwig *Anisolabis maritima*, and also with

FIGURE 1.—Model chromosome set of an autotetraploid showing homologous ends in close proximity.

DARLINGTON's (1958) statement that "The chromosomes usually begin to pair near the ends, but sometimes near their centromeres."

Once initiated, it is envisaged that pairing proceeds serially along the chromosome. The mechanism whereby this occcurs, i.e. the short-range specific forces of pairing (RHOADES 1961), can be considered virtually independently of the questions discussed in the present paper. The present discussion is limited to pointing out a way whereby homologous chromosome regions might be brought into sufficiently close proximity for the short-range forces to act.

Evidence from autotetraploids: While the eventual pairing attained in diploid organisms is largely independent of the way in which pairing is initiated, this is not the case with tetraploids. For in tetraploids if pairing is initiated at only one point along the chromosome, then serial pairing along the entire length will always result in chromosomes associated in pairs along the entire length, i.e., in bivalent formation. However initiation at more than one site can lead to quadrivalent formation, as will be described below.

As a consequence of the chromosome attachment model a definite expectation for the frequency of quadrivalents can be given. It is assumed that in this case chromosomes are grouped with all four homologous telomeres at either end attached in close proximity. This situation is depicted in Figure 1. At either end pairing will always be two-by-two, with the pairing initiated at the A end being independent of that initiated at the B end. Then assuming, without loss of generality, that A_1 pairs with A_2 and A_3 wit hA_4, i.e., (A_1A_2) (A_3A_4) at the A end, three different pairings may be initiated at the B end. The pairing (B_1B_2) (B_3B_4) will clearly lead to the formation of two bivalents. On the other hand the two other types of pairing, *viz.* (B_1B_3) (B_2B_4) or (B_1B_4) (B_2B_3) will lead to quadrivalent pairings. The resultant configuration of the first of these two pairings is depicted in Figure 2, where the chromosomes have now been unfolded to clarify

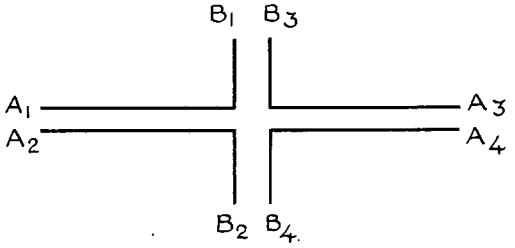

FIGURE 2.—Diagram of pairing attained in an autotetraploid, at metaphase.

the nature of the pairing, thereby upsetting the close association of all four A and all four B ends. Since all three types of pairing are equally likely in an autotetraploid, two thirds of the chromosomes will be associated as quadrivalents and one third as bivalents. For the chromosomes to maintain their association as quadrivalents until metaphase I of meiosis when observation of chromosomal structure is commonly made, it is necessary that at least three of the four arms depicted in Figure 2 be held together by chiasmata.

The expectation of a quadrivalent frequency of two-thirds when pairing is initiated at two sites has previously been put forward by HUGHES-SCHRADER (1943) and more recently by JOHN and HENDERSON (1962). The model was put forward by the latter authors in connection with the data of MORRISON and RAJHATHY (1960a,b). MORRISON and RAJHATHY studied the metaphase configurations of nineteen different tetraploid plants coming from a wide range of genera, and having a wide range of chromosome sizes. Most determinations were also made under more than one environment. In every case the frequency of quadrivalents was found to be reasonably close to the value two-thirds, the actual values ranging between 53% and 81%. Moreover, the distribution of the number of quadrivalents was found in all but one case tested to be not significantly different from expectation under the binomial distribution, thereby indicating that the frequency two-thirds was not merely a property of the average of the chromosome sets of a species but rather a property of each chromosome set of the species. In one case (*Asparagus officinalis*), a comparison was made between the long and short chromosomes within the species, and no difference in the quadrivalent frequencies was found. Similarly no differences were found between species with small and large chromosomes. On the basis of these observations MORRISON and RAJHATHY concluded that ". . . in all autotetraploids approximately two-thirds of the chromosomes form quadrivalents."

The significant deviations from two thirds observed in some species may readily be accounted for. A small negative deviation might be expected if strands fail to initiate pairings at both ends in a percentage of cases, or if sufficient chiasmata are not formed to maintain all pairings. This may be the case in *Dactylis glomerata* (MCCOLLUM 1958), perhaps the most extensively studied single tetraploid, where frequencies lower than two thirds are consistently observed. The high frequencies found by MORRISON and RAJHATHY however are difficult to explain unless pairing is initiated at more than two sites. In maize for instance, the frequency of quadrivalents found was 0.78, which is of the same order as the estimates of VENKATESWARLU (1950) and GILLES and RANDOLPH (1951). VENKATESWARLU has made a detailed study of meiosis in this species, and has described the configurations of particular chromosomes at pachytene. He finds a number of cases of two partner exchanges per quadrivalent (although none of three or more), and has interpreted these as showing initiation at three sites. No characteristic configuration of the partner exchanges was apparent, which could be a reflection of variation in either the position of pairing initiation or in the relative times of initiation at the different sites.

None of these findings are incompatible with the model put forward of pairing

initiation being favoured by telomere attachement. No special mechanism for pairing initiation has been assumed since the same type of local attraction which governs the serial pairing of chromosomes could account for the initiation. Thus while pairing is usually initiated at two sites, initiation at a third or fourth site is by no means precluded. Pairing in the median regions could be considered as a race between the serial pairing process started in the distal regions and the possibility of chance meeting of homologous regions initiating a new pairing. Clearly in some cases, such as pairing in an inversion-carrying diploid, more than two initiations of pairing are needed to explain the types of pairing found. In such a case pairing might be initiated normally near the ends, but the serial pairing process interrupted leaving unpaired regions which could subsequently pair. The pairing in the inversion might be initiated near the centre where homologous regions are closest together.

JOHN and HENDERSON (1962) studied tetraploid cells from a number of insect species and obtained quite a different picture from that in plant material. A marked tendency was found for large chromosomes to be associated as quadrivalents and small chromosomes as bivalents. Moreover, the extreme clarity of preparation possible in this material enabled the nature of the quadrivalents to be closely studied, and some quadrivalents were found with several, i.e., up to five to six partner exchanges, as opposed to only one such exchange predicted under the two-site initiation model.

While JOHN and HENDERSON claim that these results contradict those of MORRISON and RAJHATHY, this does not necessarily seem to be the case. The results from the small insect chromosomes, where insufficient chiasmata are formed to maintain quadrivalent pairings even if these are initiated, are to be expected under any model of pairing and provide no evidence on the manner in which pairing is initiated. In addition, WHITE (1954) has pointed to the fact that bouquet formation which presumably aligns long regions of homologous chromosomes before pairing begins, may be a feature of all animal meioses. Under these circumstances initiation at many more than two sites may be expected. Thus the dissimilar results of MORRISON and RAJHATHY, and JOHN and HENDERSON, could well be a reflection of distinctly different types of chromosome organization in premeiotic stages of plant and animal species. This would not be unexpected if a more complex type of organization had evolved in animal cells to ensure a higher probability of complete chromosome pairing than given by just telomere attachment.

It might be noted that while there appear to be few exceptions to the rule that newly produced autotetraploid plants show a large amount of quadrivalent formation, *Lotus corniculatus* appears to be an example of a naturally occurring tetraploid showing tetrasomic genetical behavior but having a low frequency of quadrivalents (DAWSON 1941). It seems therefore that natural selection in an autotetraploid might tend to favor the production of a genotype with almost complete bivalent formation to stabilize the chromosome behavior. In fact, the decline in the frequency of quadrivalents over a period of some generations found in autotetraploid *Zea mays* and *Brassica campestris* (GILLES and RANDOLPH 1951;

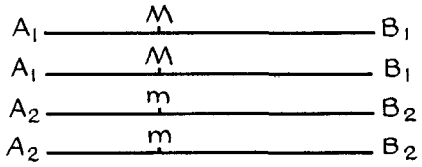

FIGURE 3.—Model chromosome set of a newly synthesized allotetraploid.

SWAMINATHAN and SULBHA 1959) may illustrate the action of this process.

Allotetraploids: In addition to testing the model on cytological data in autotetraploids, some predictions on the nature of preferential pairing in allotetraploids may be made. In particular, the expected relationship between cytological and genetical data may be derived.

A diagram of one set of the chromosome complement of a newly synthesized allotetraploid is given in Figure 3. The two initiation sites may be assumed to occur at any point of the chromosome in the following argument provided pairing is initiated independently at the two sites. The gene configuration at the *M* locus does not affect the pairing relationships. The position of the locus is also not important in the following argument.

Two types of pairing may be initiated at the first site, *viz.* (A_1A_1) (A_2A_2), with probability $1-a$, and (A_1A_2) (A_1A_2) with probability a. The parameter b may similarly be assigned to specify pairing at the second site. a and b may take any value from zero, as for very distantly related ancestors ,to two thirds, expected for closely related ancestors. It is assumed that sufficient chiasmata are formed so that quadrivalents do not fall apart. Evidence that this assumption may not be unrealistic, even for inter-genomic pairing, is given by the findings of RILEY (1960a) in nullisomic wheat. The evidence of increased amounts of inter-genomic pairing, and approximately normal chiasmata frequencies, in the absence of chromosome 5B, has been interpreted as showing that the inability of homeologues to pair rather than to form chiasmata is the limiting factor in determining their genetical behavior. The same evidence also shows that the values of a and b in any particular case are determined not only by the structure of the particular chromosomes but also by the background genotype and environment.

In a proportion $(1-a)(1-b)$ of cases bivalents with like pairing, i.e., intragenomic pairing, will be formed. All gametes arising from this configuration will have the genotype *Mm*. In a proportion, $\frac{1}{2}\ ab$ of cases bivalents with unlike, i.e., inter-genomic, pairing will be formed. One quarter of the gametes arising from such pairing would be *mm*. The remaining $a + b - (3/2)\ ab$ of cases would have quadrivalent pairing. Approximately one-sixth *mm* gametes may be expected in this case assuming random chromosome disjunction.

The total frequency of quadrivalents under the model is therefore $a + b - (3/2)ab$, while the frequency of recessives expected is $(1/6)a + (1/6)b - (1/8)ab$. These values define a family of closely related curves relating the frequency of quadrivalent formation and the frequency of recessive segregants.

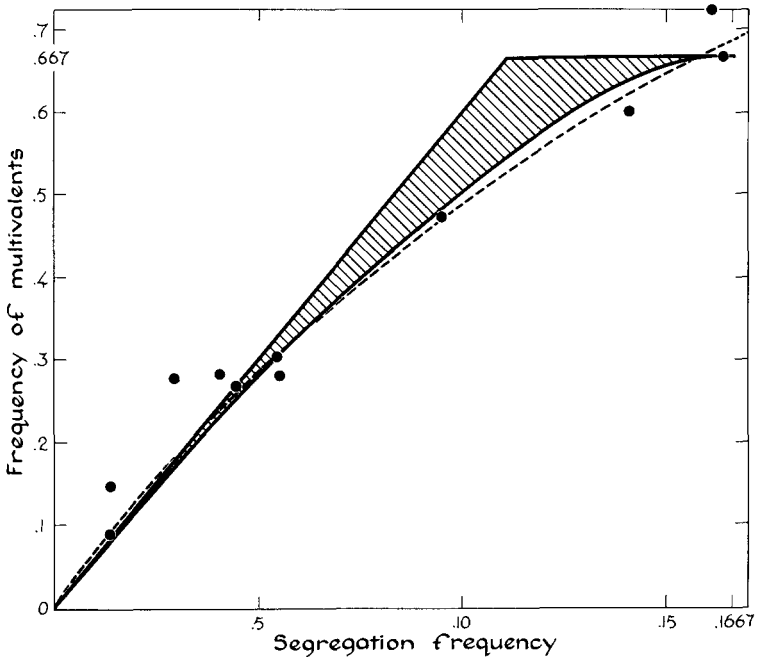

FIGURE 4.—Expected relationship between cytological and genetical data in allotetraploids, fitted to data of PHILLIPS (1964). The broken line is the regression line given by PHILLIPS (1964).

These are given in Figure 4, where the shaded area defines the possible range of values of the two frequencies under the model.

Data on segregation and quadrivalent formation in allopolyploids of the genus Gossypium (see PHILLIPS 1964 for summary) may be used to test these expectations. Nearly all points are seen to lie close to the curves, while the range of values is almost exactly that predicted by the model. PHILLIPS has argued generally that the relationship between the two variables would be a curvolinear one, and has fitted a best-fit quadratic regression line. This is seen to be almost coincident with the values given under the two-site initiation model when $a = b$, which might be approximately expected in practice.

Further evidence comes from a study made by RILEY (1960b) of the relative positions of homeologous chromosomes on the metaphase plate in hexaploid *Triticum aestivum*. A marked tendency for homeologous chromosomes to lie close to each other was found in this species where only homologous chromosomes regularly pair with each other. Such secondary pairing, as this has been described, might be expected even if homeologues rarely pair, since the postulated attachment could lead to a close association throughout meiosis.

Inversion-carrying tetraploids: The results from experiments of DOYLE (1963) in inversion-carrying tetraploid maize are seen to be compatible with the scheme of telomere attachment. An inversion introduced close to one end of chromosome 2 resulted in a reduction in the frequency of recessive segregants from a duplex backcross. Telomere attachment in this case would tend to align nonhomologous

portions of the inversion over a long distance, and thus encourage preferential pairing at one end. Together with random pairing at the other end, and possibly some pairing initiation in the median regions, this would be sufficient to explain the observed deficiencies of recessive gametes from tetrasomic expectations. A more critical test of the model could perhaps be made with combinations of suitably chosen inversions particularly in the distal regions.

E. H. GRELL (1961) has undertaken a similar experiment in triploid Drosophila, using stocks carrying a number of inversions. While the interpretation is complicated in this case by the possible loss of chromosome fragments and univalents, it appears that the inversions do not have a markedly large effect until nearly all of the chromosome is involved. This would not be unexpected if, as postulated previously for the insect species, not merely the telomere regions but most of the lengths of homologous chromosome are aligned before pairing begins.

DISCUSSION

Some discussion of the possible origin and nature of the chromosome-nuclear membrane association seems desirable. First it seems possible that the attachment is not limited to those chromosomes about to undergo meiosis, but that the close juxtaposition of homologous telomeres is brought about in the zygote immediately following fertilization. This could be achieved by fusion of the two gametic nuclei occurring with the two nuclei oriented in a completely specified manner. Possible indications that the attachment is not limited to meiotic chromosomes come from the observations of SCHNEIDERMAN and SMITH (1962) and others in human mitotic chromosome material. It has been shown that homologous chromosomes tend to lie closer to each other on the metaphase plate than expected by chance, which could be a residual effect of the close attachment of homologues. Also the finding of somatic pairing and occasional mitotic crossing over in a number of species suggests the existence of a mechanism for pairing initiation in mitotic as well as meiotic chromosomes.

If the nuclear membrane remained intact during mitosis, some difficulty might be felt in envisaging a way in which attachments of sister chromatids could pass to opposite poles at anaphase. Since the nature and extent of the breakdown of the membrane and also its reconstitution are somewhat obscure (MAZIA 1961), there seems little point at present in speculating on the manner in which the attachment or the close juxtapositions of homologous telomeres could be conserved. It is possible that a direct attachment might not at all times be required, provided that the attachment could be maintained by fibres to ensure that contact is not lost.

One difficulty which the postulation of telomere attachment might overcome is in explaining the low frequency with which interlocking bivalents and quadrivalents are found. Regardless of the accuracy of the model in predicting quadrivalent frequencies it is seen that the production of quadrivalents in tetraploids indicates that pairing is initiated at more than one position on the chromosome.

If this is the case, then if chromosomes are free-floating, interlocking of different chromosome sets might occur frequently both in diploid and tetraploid organisms.

One or two other genetical results and hypotheses may be mentioned in relation to the hypothesis of telomere attachment. First such attachment, if arising immediately following fertilization, could give rise to a phenomenon similar to genetical affinity (WALLACE 1953 *et seq.*), especially of such a type as postulated to occur in some Gossypium hybrids (WALLACE 1960). It must nevertheless be noted that several cases have been reported of chromosomes from different parents segregating preferentially together, which could not be simply explained on the attachment hypothesis. Secondly, it has been suggested (BODMER 1965) that chromosome replication might take place with homologous chromosomes associated at a common replicating point on the nuclear membrane. Finally it is interesting to note that two recent reports have invoked a spatial organization of chromosomes in early meiosis to explain data quite different from those on which the present suggestion is based. OKSALA (1958) has proposed an attachment in Drosophila very similar to that proposed in this paper, while NOVITSKI (1964), in explaining data of R. F. GRELL (1962) also in Drosophila, has suggested that a definite arrangement of heterologues is found, which might also be explicable in terms of attachment to the nuclear membrane.

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SUMMARY

Starting with the assumption of adjacent attachment of homologous telomeres to the nuclear membrane, which seems plausible on the basis of several studies cited, a number of genetical and cytological consequences in tetraploid organisms are derived. It is shown that if pairing is initiated in the distal regions as a result of the attachment, then approximately two thirds of the chromosomes of an autotetraploid may be expected to associate in quadrivalents at metaphase, a result previously given by several authors. This expectation is seen to accord well with the data of MORRISON and RAJHATHY (1960a,b) over a wide range of plant species. The relationship expected between cytological and genetical data in allotetraploids may also be derived, and a comprehensive set of data in the genus Gossypium (PHILLIPS 1964) is seen to give good agreement. Finally in inversion-carrying tetraploids a reduction in the frequency of segregation comparable to that found by DOYLE (1963) is shown to be expected.

It is pointed out that the attachment postulated provides a possible answer to the question of why interlocking of bivalents in diploids at meiosis is rarely found. It is suggested that such attachment may be a property of chromosomes throughout the life cycle, and could possibly arise in the zygote following fertilization as a consequence of the orientation of the fusing nuclei.

Note added in proof: Micromanipulation and centrifugation studies by PUSA (1963) which strongly suggest the association of chromosome and nuclear membrane were earlier overlooked

by the author. KASHA and BURNHAM (1965; see also earlier references) have recently reported results of experiments utilizing combinations of interchanges in opposite chromosome arms which support the hypothesis that pairing is initiated in the distal regions of the chromosome.

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